PHYSIOLOGICAL ZOÖLOGY

A Quarterly Journal of Zoölogical Research

CHARLES MANNING CHILD AND WARDER C. ALLEE
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LOCALIZED STIMULATION, TRANSMISSION OF IMPULSES, AND THE NATURE OF RESPONSE IN AMOEBA[1]

(Three figures)

S. O. MAST
The Johns Hopkins University

INTRODUCTION

ACCORDING to Bütschli's surface-tension theory of locomotion (1892), response in *Amoeba* is the result of local reduction in surface tension and there is nothing in the nature of what is ordinarily called an "impulse." Rhumbler (1898) and many others support this view.

McClendon (1909) maintains, however, that localized chemical stimulation of one pseudopod of an amoeba results in cessation of movement in all the pseudopods, that is, that such stimulation produces impulses which are transmitted to all parts of the body; and he asserts that the rate of transmission of these impulses is greater than 0.03 mm. per second. And Verworn (1913) comes to similar conclusions in regard to *Difflugia* stimulated by contact.

I observed (1910) in amoebae traveling toward a spot of well-defined, intense light on a slide that movement ceases at the anterior end almost immediately after the tip of this end has become illuminated, and that this cessation spreads, under certain conditions, especially in monopodal specimens, through the entire organism.

[1] Contribution from the Mount Desert Island Biological Laboratory. I am greatly indebted to the director, Professor H. V. Neal, for laboratory facilities, to the Research Corporation for financial aid, and to C. Ladd Prosser for very efficient assistance.

I also observed that after an amoeba has been brought to rest by stimulation of the anterior end it soon becomes active again, and that under certain conditions this manifests itself by the formation of a pseudopod at the posterior end. These facts seem to show that stimulation of the tip of advancing pseudopods results in the production of impulses which travel through the entire body. They are therefore in accord with McClendon's views.

Edwards obtained similar results with acids. He says (1923, p. 8) that if HCl N/1 is applied to the advancing anterior tip of an amoeba, "there is almost immediately an eruptive forward flow followed by cessation and reversal of streaming" and that "a pseudopod then forms at the posterior end or laterally." And he asserts that if the acid is applied locally to the side of an advancing pseudopod some distance from the tip, new pseudopods form "directly opposite the region stimulated."

These results lead to the same conclusion regarding impulses as those which I obtained with photic stimulation. There is, consequently, considerable evidence at hand which indicates that localized stimulation in *Amoeba* results in the production of impulses which travel to distant parts of the body and produce action there. Is this actually true? If so, what are the processes involved in localized stimulation, in the transmission of impulses, and in the reactions produced? In the following pages we shall consider the bearing upon these questions of the results obtained in a study of the response to localized photic stimulation in various amoebae.

MATERIAL AND METHODS

Four distinct types of amoebae were used in the following observations: namely, *Amoeba proteus* (L), *Amoeba dubia* (S), *Amoeba dofleini* (N), and a small unidentified form which I shall designate *Amoeba X*.[1] The first three were raised in the laboratory; the last was obtained from a lake some distance from the laboratory.

[1] *Amoeba X* was found in July in Graham Lake, Ellsworth, Maine. It was fairly abundant on aquatic vegetation and organic débris near the shore. The water in this region was slightly acid, pH 6.8. This amoeba has the following characteristics:

Form: Usually monopodal, anterior end considerably larger than posterior.

Size: About 0.165 mm. long during active locomotion.

Structure: One contractile vacuole; one spherical nucleus with a layer of prominent

The light used to localize the stimulus consisted of the image of the luminous filament of a monoplane-filament, stereopticon lamp. The image was produced by means of the substage condenser on a binocular mono-objective microscope. The light from the lamp was reflected up through the condenser by means of the plane substage mirror. The image thus produced could readily be moved in any direction in the plane of the stage by rotating the mirror, and it could be adjusted vertically in reference to the surface of the stage by raising or lowering the condenser. In most of the tests the lamp was so screened that only a portion of one section of the filament was exposed. The image of this portion was rectangular, 0.025 mm. wide and 0.1 – mm. long. The edges were sharp and clear cut. There were, however, secondary images some little distance on either side of the image; but the luminous intensity of these was, in relation to that of the primary image, so low that they did not appreciably interfere.

In making the observations several amoebae were mounted on a slide under a cover glass sealed with vaseline. They were then put onto the stage of the microscope and adapted to low illumination, after which a specimen was selected and the image of the luminous filament brought to a sharp focus on it by adjusting the condenser. Then the image and the amoeba were moved about, the one by means of the substage mirror and the other by means of the mechanical stage, so as successively to illuminate various portions of the organism.

The observations were made with 10×dry, 20×dry, and 60×oil immersion objectives and 20×compensating oculars, that is, under magnifications of 200, 400, and 1,200 diameters, respectively. By means of this apparatus and these methods, the stimulus was very precisely localized and the effect was clearly seen.

RESULTS

The results obtained were essentially the same for all of the types studied except *Amoeba dofleini*. Those obtained with *Amoeba X*

granules at the surface surrounding a large hyaline central portion; about fifteen prominent flat, diamond-shaped crystals; hyaline cap, usually large and well differentiated.

Action: Locomotion rapid, response to light very definite.

were particularly definite. I shall therefore present them first; but before describing the responses in this form to localized stimulation, it will be expedient briefly to consider the responses to changes in the illumination of the entire organism. These were observed as follows:

Several specimens were mounted on a slide on the stage of the compound microscope, as previously described. An active individual was then selected and adapted to moderate illumination, after which it was subjected to increase and to decrease in illumination by opening and closing the substage diaphragm. The results obtained in numerous tests lead to the following conclusions:

If the illumination is decreased, there is no appreciable response; but if it is increased, there is a very definite response, the nature of which depends upon the extent of the increase. If the increase is marked, all streaming ceases. Cessation always begins at the anterior end immediately back of the hyaline cap and then spreads rapidly backward; but if the increase is not too great, streaming soon begins again. If the increase is moderate, it begins first at the anterior end and then spreads rapidly toward the posterior end until the entire plasmasol is involved. If it is rather extensive, streaming begins at the posterior end but in the reverse direction. This reversal may be only temporary and confined to the posterior portion of the organism, or it may be permanent and extend throughout the entire plasmasol, the posterior end becoming permanently transformed into an anterior end. If the reversal is temporary and confined to the posterior end, streaming may begin at the anterior end before the reversed streaming ceases at the posterior end, resulting in currents in opposite directions at the two ends. All this also obtains, under certain conditions, if the intensity is decreased shortly after it has been increased. This is in accord with results obtained by Folger (1925).

Let us now consider the results obtained with localized stimulation of *Amoeba X*. If the hyaline cap only is illuminated, there is no response (Fig. 1*A*). This shows that the hyaline cap is not photosensitive.

If the hyaline cap and the tip of the plasmagel sheet are illuminated, forward-streaming stops at the anterior end and two lateral

pseudopods develop near the tip, one on either side. These frequently continue until the amoeba is stretched out along the edge of the luminous field (Fig. 1*B*). This shows that the plasmagel sheet is photosensitive.

If one side of the plasmagel sheet is more intensely illuminated than the other, streaming continues, but the anterior end is deflected toward the opposite side (Fig. 1*C*). This indicates that light affects

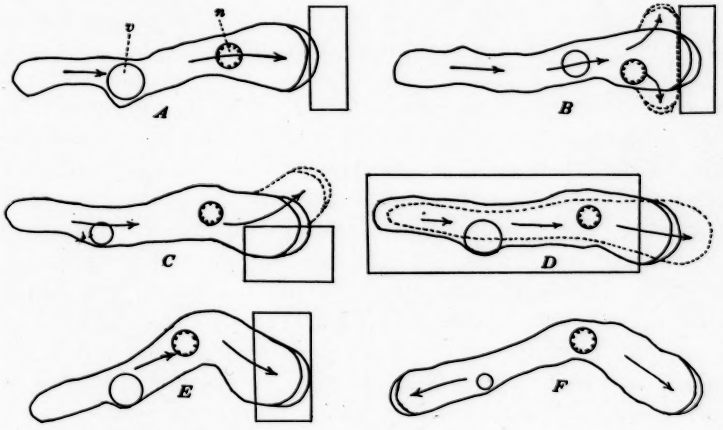

FIG. 1.—Camera drawings of *Amoeba X*, illustrating the response to localized illumination. Rectangular areas, regions of high illumination; arrows, direction of protoplasmic streaming; dotted outlines in B, C, and D, positions and forms shortly after the illumination of the parts indicated; *n*, nucleus; *v*, contractile vacuole. *E* and *F*, same specimen; *F*, form and direction of streaming assumed by *E* after the anterior end had been illuminated for a few moments.

directly the region illuminated and that this effect is not transmitted to regions not illuminated.

Illumination of the body back of the plasmagel sheet, no matter how much, does not produce cessation in streaming. This seems to show that the photosensitive substance is confined to the plasmagel sheet, i.e., that this sheet is the photoreceptor in *Amoeba*. This is, however, not true, for when the posterior portion of the body is illuminated, the rate of streaming in the plasmasol increases and the amoeba becomes thinner and longer (Fig. 1*D*). The fact that

the rate of streaming increases indicates that the posterior portion of the body, as well as the anterior portion, is sensitive to light, and the fact that the body becomes thinner and longer indicates that light causes contraction of the plasmagel in the region illuminated.

If the anterior end extending back to some little distance beyond the plasmagel sheet is illuminated (Fig. 1*E*), streaming at the anterior end ceases, then cessation spreads until the entire organism comes to rest, after which streaming begins at the posterior end but in the reverse direction. If the illumination is decreased immediately after streaming has begun in the reverse directions at the posterior end, it beings at the anterior end and proceeds in the original direction, resulting in streaming in opposite directions at the two ends (Fig. 1*F*). This, however, continues only a short time, after which the direction of streaming at the posterior end reverses again and then streaming proceeds in the original direction throughout the entire plasmasol. This supports the contention that *Amoeba* is a turgid system; and it indicates that light causes an increase in the elastic strength of the portion of the plasmagel illuminated, that during locomotion the elastic strength of this layer is lower at the anterior than at the posterior end and lower at the posterior end than in the middle, and that by local illumination of the anterior end it can be increased until it is greater here than at the posterior end.

All of the results obtained with localized illumination are in accord with the contention that light increases the elastic strength of the portion of the plasmagel illuminated. Cessation of streaming produced by increase in the illumination of the entire organism is doubtless due to proportionally greater increase in the elastic strength of the plasmagel at the tip of extending pseudopods than elsewhere. This conclusion is strongly supported by the fact that cessation in streaming always begins at the tip of such pseudopods and by the fact that under certain conditions it begins at the posterior end after recovery, indicating that the elastic strength at the anterior end was augmented by the action of the light until it became greater than that at the posterior end.

The increase in the elastic strength of the plasmagel is probably due to increase in the thickness of this layer produced by gelation of the adjoining plasmasol, owing to the action of the light. The

results obtained in observations made on *Amoeba proteus* lead essentially to the same conclusion.

In the observations on *Amoeba proteus* several specimens on a slide were subjected to low illumination for 10–15 minutes, then one of the specimens was selected and the relative thickness of the plasmagel and the plasmasol of the central portion of the body measured by means of a camera lucida and the form of the body noted, after which all but a small portion at the anterior end was subjected to high illumination by focusing on it the image of a piece of ground glass in close contact with the stereopticon lamp used in the preceding tests. This illumination was continued 3–5 minutes, after which the relative thickness of the plasmagel and the plasmasol of the central portion of the body was again measured and the form of the body again noted.

During this illumination the amoebae studied usually became markedly longer and thinner (Fig. 2*B*). In some specimens the part illuminated became thinner, while that not illuminated spread out, forming a bulb-like enlargement (Fig. 2*A*). In all, the plasmagel became thicker in relation to the plasmasol in the region illuminated. This is shown in the camera drawings reproduced (Fig. 2), but it is more conclusively demonstrated by the averages of the results obtained in all the measurements made.

Four individuals were used, and the thickness of the plasmagel and of the plasmasol was measured, as described above, four times in each individual, twice in low and twice in high illumination. The gel/sol ratio of the average of the results obtained in high illumination was 1.45−, and that of the results obtained in low illumination was .78−. This indicates that in relation to the plasmasol the plasmagel nearly doubled in thickness during the exposure to high illumination although the actual increase in thickness of the plasmagel was slight. The change in form, especially the decrease in thickness of the highly illuminated portion and the enlargement of the remainder of the body, shows that the portion illuminated contracted; and it indicates that the light caused increase in the elastic strength of the plasmagel, which forced the plasmasol forward, resulting in expansion of the anterior end.

The conclusion that the gelating effect of light, resulting in

increase in the elastic strength of the portion of the plasmagel illuminated, is not transmitted to adjoining regions is strongly supported by results obtained with multipodal specimens of *Amoeba proteus* and *Amoeba dubia*, especially the latter.

McClendon (1909) maintains, as previously stated, that chemical stimulation of one pseudopod in multipodal amoebae causes cessa-

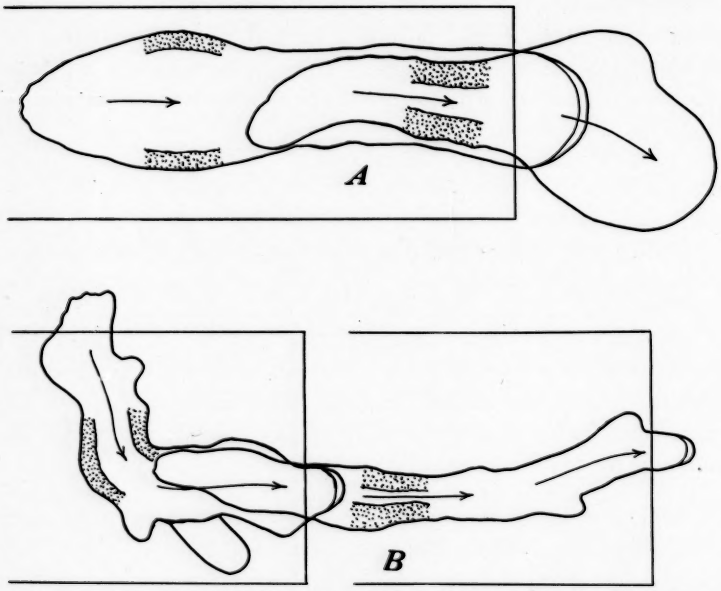

FIG. 2.—Camera drawings of *Amoeba proteus*, illustrating the effect of illuminating all but the anterior end. Rectangular inclosures, regions of high illumination; arrows, direction of protoplasmic streaming; stippled regions, plasmagel, indicating thickness in relation to the plasmasol.

A, change in the position and in the form of a specimen with the illuminated region fixed. Note that as this specimen moved forward, the illuminated part contracted and the shaded part expanded. *B*, change in the position and the form of a specimen with the illuminated region moved forward as rapidly as the anterior end of the amoeba moved forward. Note that the specimen elongated greatly and that the plasmagel became relatively much thicker.

tion in streaming and retraction in all the pseudopods, indicating that localized stimulation produces impulses which travel throughout the body.

Amoeba X is continuously monopodal or nearly so. It is therefore not so well adapted for the investigation of the problem concerning transmission as are *proteus* and *dubia*, which usually have two or more active pseudopods. In making observations on these forms, an active multipodal specimen was selected; then a portion of one of the advancing pseudopods was illuminated, as described above. The results obtained were the same in both forms.

Illumination of the anterior portion of any active pseudopod produced cessation in streaming in this pseudopod. This cessation al-

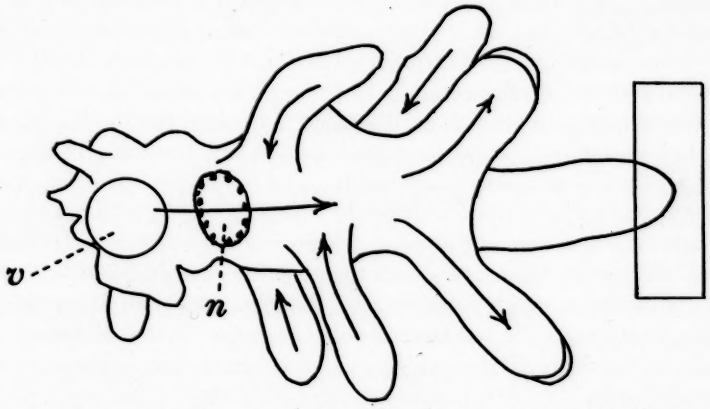

FIG. 3.—Camera drawing of *Amoeba dubia*, illustrating the effect of illumination of the tip of an actively extending pseudopod. Rectangular area, region of high illumination; arrows, direction of streaming; *n*, nucleus; *v*, contractile vacuole. Note that streaming stopped in the pseudopod illuminated, but not in any of the others.

ways began at the tip, and under some conditions it was confined to the tip and continued only a few moments, after which streaming was resumed; but under others the entire pseudopod illuminated came to rest. There was, however, no indication of cessation or retardation in streaming in any of the other pseudopods, no matter how extensive the illumination was, provided it was confined to one pseudopod (Fig. 3). These results strongly support the conclusion reached in the observations on *Amoeba X*, namely, that the gelating effect on the plasmagel of localized stimulation is not transmitted.

In *Amoeba dofleini* under some conditions, results were obtained which are in full harmony with those presented in the preceding pages; but under other conditions the results were radically different. This was especially true for specimens taken from an old culture in which the soluble content had become considerably concentrated, owing to evaporation of water.

Specimens taken from this culture were definitely positive to moderate illumination. When the image of the filament of the stereopticon lamp was brought near them on the slide, they invariably went toward it, and eventually directly into the illuminated region of the image; and when this image was brought onto them, there was no observable retardation in protoplasmic streaming, no matter what portion was illuminated. However, if the entire organism was illuminated with a much more intense light than that in the image of a filament of the lamp, it caused cessation in streaming, although the response was never very definite and it required much greater increase in illumination to produce it than is required for the other types studied. *Amoeba dofleini* is therefore, at least under certain conditions, positive in moderate and negative in strong light. There is no evidence which indicates that this obtains for the other types of amoebae studied; but there is some evidence which indicates that one of these types, *Amoeba proteus*, is sometimes photo-positive in very weak illumination. This has been observed a few times in our laboratory, and the positive reactions to bits of glass described by Schaeffer (1916) probably were positive reactions to the light reflected from them. The difference in the response in *Amoeba dofleini* and the other types mentioned is therefore probably quantitative rather than qualitative. But how are the photo-positive responses to be explained?

If photo-negative response is due to gelation of the plasmasol in the region illuminated and photo-negative orientation in lateral illumination to greater gelation on the more highly than on the less highly illuminated side, one would expect photo-positive response and orientation to be due to the opposite, i.e., one would expect, in very weak lateral illumination, solation on the more intensely illuminated side or gelation on the shaded side or both; and one would expect increase in illumination to produce solation in place of gela-

tion. There is, however, no direct evidence indicating that this actually obtains.

DISCUSSION

Jennings (1904), McClendon (1909), Mast (1910), and Edwards (1923) observed that localized stimulation results in the formation or the retraction of pesudopods at some distance from the region stimulated. If the conclusion reached above is correct, namely, that local gelation or solation in *Amoeba*, produced by local stimulation, does not result in the formation of impulses which produce gelation or solation in other regions, how is it possible to explain these results?

Jennings (1904, p. 189) maintains that if strong solutions of acids, alkalies, salts or non-electrolytes are brought in contact with one side of an amoeba, new pseudopods form directly opposite. McClendon (1909), as previously stated, contends that if acid is applied to one of several advancing pseudopods streaming stops in all. Edwards (1923) asserts that if acid is applied to a small area on one side of a pseudopod some distance from the tip, a new pseudopod forms directly opposite.

Jennings observed that the surface of an amoeba to which strong solutions of chemicals had been applied contracts. This contraction is doubtless due to increase in the elastic strength of the plasmagel, owing to gelation of the adjoining plasmasol. If an amoeba is a turgid system, contraction of the plasmagel in one region must result in expansion and the formation of a protuberance in another. We consequently see how gelation produced by the application of chemicals to one side of an amoeba could, without change in the consistency of the substance in other regions, result in the formation of the pseudopods on the opposite side observed by Jennings. This does not, however, account for the results obtained by McClendon, and it probably does not account for those obtained by Edwards.

Edwards confirmed Jennings' observations concerning the effect of local application of strong solutions of acids, but he also demonstrated that if the solution is very weak, a pseudopod is formed at the place of application.

The formation of pseudopods observed by Edwards directly opposite the region toward which acid was directed was probably due to a slight increase in the hydrogen-ion concentration at the surface

where these pseudopods formed, owing to diffusion. This is, I think, precisely what would be expected under the conditions of the experiments, for the acid used was strong enough to prevent formation of a protuberance on the side of the pseudopod to which it was directly applied; and acid of this strength would doubtless in a very short time, owing to diffusion, become strong enough on the opposite side to produce a protuberance there.

By assuming, then, that a weak solution of acid results in solation and decrease in the elastic strength of the plasmagel, we can readily account for the phenomena observed by Edwards without postulating production of impulses which result in gelation or solation at a distance from the region stimulated.

The cessation of streaming observed by McClendon in pseudopods which were some distance from the one toward which acid was directed was probably also due to diffusion of the acid, for this is precisely what would be expected on the basis of Edward's results if the acid which reached the distant pseudopods was sufficiently concentrated; and, judging from McClendon's description, it may well have been sufficiently concentrated.

I found in *Amoeba proteus* traveling toward a highly illuminated field that as soon as the tip of an advancing pseudopod enters the field it stops, but that the organism retains its direction of locomotion until several pseudopods, one after another, have come in contact with the field of light and have been stopped, when the direction of streaming in the central part of the body reverses and a pseudopod develops at the posterior end. That is, that after several successive responses to given localized stimulation there is a radical change in the nature of the response which under the circumstances is adaptive and seems to indicate that there is in this, the simplest of our animals, a process which resembles what is ordinarily called "learning" in higher organisms.

In the experiments described in the preceding pages, I observed in *Amoeba X* that if the anterior end is strongly illuminated, streaming reverses in a similar manner and a pseudopod forms at the posterior end; and I have repeatedly observed the same in *Amoeba proteus* after subjecting the entire organism to strong light for a short time.

Under these conditions, then, reversal in the direction of stream-

ing and the formation of pseudopods is obtained with a single stimulus, whereas under the conditions which obtained in the observations on specimens traveling toward a field of light, it was obtained only after a series of stimuli, each followed by a response. Under the former conditions, however, the light energy received by the organism during the period of stimulation was much greater than that received during the period of a single stimulus under the latter conditions, but in all probability not so great as that received during the series of stimuli which resulted in reversal. This seems definitely to indicate that the reversal under the latter condition was due to the cumulative effect of repeated stimulation. We have demonstrated fairly conclusively that reversal under the former condition is due to increase in the elastic strength of the plasmagel at the anterior end, owing to gelation of the plasmasol in this region induced by the light, to such an extent that the elastic strength of the plasmagel at this end is greater than at the posterior end. The same is probably true under the latter condition. If it is, we have here a fairly comprehensive insight into a process which closely resembles what is usually called "learning."

The evidence presented in the preceding pages indicates that, while gelation or solation produced in a pseudopod of an amoeba by localized illumination is not followed by gelation or solation in other pseudopods, the effect of localized gelation and solation is transmitted, that *Amoeba* acts as an organized unit, and that its actions are, on the whole, adaptive.

The nature of this transmission through the organism is evident. The plasmagel constitutes an elastic sac which, owing to the turgidity of the organism, is under tension. Local gelation causes local increase in the thickness and in the elastic strength of this layer. This results in contraction of this layer in the region stimulated which, owing to the turgidity of the organism, causes expansion and bulging in other regions. The action at a distance from the region stimulated is, therefore, merely the result of transmission of pressure.

SUMMARY

Increase in the illumination of the hyaline cap in *Amoeba* has no effect.

Increase in the illumination of the plasmagel sheet produces cessa-

tion in streaming at the tip, and results in the formation of lateral pseudopods near the tip.

Increase in the illumination of the anterior end to a point some little distance back of the plasmagel sheet causes cessation in streaming throughout the entire organism.

Increase in the illumination of one side of the plasmagel sheet causes deflection in the direction of streaming toward the opposite side.

Increase in the illumination of any portion of the posterior part of the body up to a point a little distance back of the plasmagel sheet augments the rate of streaming and causes the animal to become thinner and longer.

Increase in the illumination of one pseudopod in multipodal specimens causes cessation in streaming in that pseudopod, but it has no appreciable effect on other pseudopods.

Light causes gelation of the plasmasol adjoining the plasmagel, making it thicker and increasing the elastic strength of the portion illuminated. This effect of light is not transmitted to other regions of the organism. There are no impulses produced.

Photic stimulation consists in differential localized increase in the elastic strength of the plasmagel, and response to light is due to contraction of the plasmagel in the region stimulated, owing to the increase in the elastic strength of the plasmagel in this region.

The gelating effect of light is not transmitted, but the effect of localized gelation and the consequent contraction is transmitted. It causes expansion in all regions of the plasmagel in which the elastic strength is lower than that in the region stimulated.

Amoeba is a unified, co-ordinated system.

The so-called "process of learning" in *Amoeba* consists of the cumulative gelating effect of successive localized illuminations. The same response can be produced with a single illumination if the light is intense enough and the period of illumination is long enough.

Our knowledge concerning the nature of stimulation, transmission, and response in *Amoeba* is probably more comprehensive than it is for any other organism.

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THE SENSITIVITY OF THE LEGS OF CERTAIN CALLIPHORIDAE TO SACCHAROSE

(One figure)

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I. INTRODUCTION

DURING recent years there has been a growing interest in the sense of taste in insects. The mouth parts of insects have been rather thoroughly studied from a morphological viewpoint, but from a physiological viewpoint much remains to be done. Will (1885) and Forel (1908) were among the first investigators in the physiological field. In recent years, however, von Frisch (1927, 1928, 1930) and Kunze (1927) have added important contributions in their work on the sense of taste in the honeybee.

The organs of taste in insects, however, are not confined to the mouth parts. Minnich (1921) noticed that certain nymphalid butterflies, in walking over a substratum, thrust out their proboscides whenever they encountered food materials. Experiments showed that the receptors which effected this response were located in the walking legs. Further studies on these butterflies (Minnich, 1922*a*, 1922*b*) and on certain muscid flies (Minnich, 1926, 1929) have shown the following facts pertaining to the chemoreceptors of the legs. First, both the butterflies and the flies used could distinguish a 1-M saccharose solution from distilled water by means of their legs. Second, the butterflies, as well as the flies, experienced a fall in the threshold of response to saccharose during a period of water diet lasting until death. In some cases a threshold between $\frac{1}{25,600}$ M and $\frac{1}{6,400}$ M was reached in the last stages of water diet. Third, sugars were perceived in the following order of effectiveness by the legs of *Calliphora vomitoria:* saccharose (=)[1] maltose > fructose > glucose > lactose. Lactose was wholly ineffective for the legs of the fly. Von

[1] Approximately equal.

Frisch (1928) had previously shown this to be true for the mouth parts of the honeybee.

Verlaine (1927) has recently published a series of experiments on the cabbage butterfly, *Pieris rapae* Linn., using methods somewhat similar to those of Minnich. He was not able to get any clear evidence of chemical discrimination on the part of the tarsi, however, and concluded that *P. rapae* did not possess chemoreceptors in its tarsi. Furthermore, he questioned the validity of Minnich's conclusions in the work on nymphalid butterflies. In his last work on the blowfly, however, Minnich (1929) has again brought very critical evidence showing clearly that some insects do possess organs of taste in their legs.

It is the purpose of this paper to present data on the thresholds of response to saccharose in the legs of several common blowflies. The thresholds have been studied daily during periods of water diet lasting until death.

The writer wishes to acknowledge her indebtedness to Dr. D. E. Minnich for suggesting the problem and for constructive criticism made during the progress of its study.

II. CULTURE METHODS

Four species of Calliphoridae were used in my experiments—viz., *Cynomyia cadaverina* Desvoidy, *Calliphora erythrocephala* Meigen, *Lucilia sericata* Meigen, and *Calliphora vomitoria* Linn. Dr. J. M. Aldrich, of the United States National Museum, very kindly identified the first three of these species, and Dr. D. E. Minnich verified the identification of the fourth.

Cultures were started with flies caught from baits such as fish heads and banana mash, exposed on a south window ledge. The general method used for culturing the blowflies was essentially the same as that used by Minnich (1929).

The breeding cages were supplied with 1-M saccharose solution and meat, consisting of liver, fish heads, and occasionally a fresh rodent carcass. For egg production, *Lucilia* needed meat but once a week. The other three species, however, required meat continuously. The number of eggs produced varied with the species. *Calliphora vomitoria* laid the fewest and *Lucilia* the most, while the other two

were intermediate. Of the four species, all were easily cultured through the winter except *Calliphora vomitoria*, the strain of which was lost in mid-winter.

The general behavior of the several species differed considerably. *Cynomyia* and *Calliphora vomitoria* were the easiest to handle, while *C. erythrocephala* proved to be the most difficult.

III. EXPERIMENTAL PROCEDURE

The general experimental procedure was practically identical with that used by Minnich (1929), and for a more detailed account the reader is referred to that source. This investigator showed that, while all the legs of *Calliphora vomitoria* were sensitive to saccharose, the right foreleg was more convenient to work with than the others. The flies in my work were mounted, therefore, with the right foreleg free.

Sugar solutions were mixed fresh each day from chemically pure saccharose and distilled water. The complete series of concentrations used was the same as that used by Minnich—viz. $\frac{64}{100}$ M, $\frac{32}{100}$ M, $\frac{16}{100}$ M, $\frac{8}{100}$ M, $\frac{4}{100}$ M, $\frac{2}{100}$ M, $\frac{1}{100}$ M, $\frac{1}{200}$ M, $\frac{1}{400}$ M, $\frac{1}{800}$ M, $\frac{1}{1,600}$ M, $\frac{1}{3,200}$ M, $\frac{1}{6,400}$ M, $\frac{1}{12,800}$ M and $\frac{1}{25,600}$ M. The solutions were tested in Stender dishes.

If the leg of a blowfly was brought in contact with a saccharose solution of sufficient concentration, there followed a typical response, in the form of a quick vigorous extension of the proboscis. To less effective stimuli the flies responded by extending the proboscis only partially. Still less effective stimuli failed to elicit any proboscis movement whatever.

Newly hatched flies 0–24 hours old were mounted, and 1 hour later were permitted to drink from a 1-M saccharose solution all they would. Twenty-four hours later and on each succeeding day, the legs were tested with various concentrations of saccharose to determine the exact concentration necessary to effect a proboscis extension. During this experimental period the animals were on a water diet. As a rule, each fly was given 10–15 trials with saccharose solutions

daily, each trial to saccharose being immediately preceded by a trial with distilled water, which served as a check trial. A 15-minute interval was allowed between two consecutive trials with the same individual. This is important, for Minnich noted that the chemoreceptors of the legs of *Calliphora vomitoria* were readily fatigued.

IV. RESULTS

Under the conditions of the experiment—viz., water diet following a single administration of 1-M saccharose—*Lucilia* lived from 6 to 8 days; *Cynomyia* and *Calliphora vomitoria*, from 5 to 7 days; and *C. erythrocephala*, 4 to 6 days. During this experimental period, the tendency of the various species of flies to respond to water and to exhibit adventitious proboscis movements was carefully observed. This was important, because the reliability of the results with sugar depended largely upon the absence of such responses.

Lucilia rarely responded to water. Of the 57 animals tested, only 5 responded to water in the entire series of trials. Three of these were partially responsive for only 1 day, each responding to water in but 3 of the 15 trials. The other two became more or less consistently responsive to water, one for a period of 1 day, the other for a period of 2 days. When an animal responded to water consistently, it was considered "water-responsive."

Although the condition of "water-responsiveness" was rare in *Lucilia*, it occurred often in the other three species, particularly on the last day of water diet. Thus, of the 37 *Calliphora erythrocephala* tested, 16 became water-responsive at some time or other, most of them the day before death. This response to water did not mean that the animals were always "thirsty," for they frequently did not drink. In Table I, a typical protocol is shown, in which the animal was responsive to water on the second and fifth day of water diet, but not on the third and fourth.

Of the 10 *Calliphora vomitoria* tested, 7 were water-responsive at some time during the experiment, usually at the very end of life. In general, these animals were quite consistent in their behavior; and when they responded to water, they always drank. Minnich (1929) states that one-half of his animals became more or less responsive to water for a period of variable length during the last 2

TABLE I

Showing a typical protocol of *Calliphora erythrocephala*. Symbols: o, no response; 1, feeble response; + vigorous response.

Period of Water Diet	Molarities of Saccharose															Thresholds Assigned
	1/25,600	1/12,800	1/6,400	1/3,200	1/1,600	1/800	1/400	1/200	1/100	2/100	4/100	8/100	16/100	32/100	64/100	
Second day, 23–27 hours	Water-responsive															
Third day, 52–56 hours							o o o	o 1	o +	+ + + +	+					1/400 M.–2/100 M.
Fourth day, 72–76 hours							o o o	+ + + +	1	+						1/400 M.–1/200 M.
Fifth day, 98–102 hours	Water-responsive															
Sixth day, 124–28 hours	Moribund															
Seventh day, 146–48 hours	Dead															

days of life. Some of these remained negative enough to water to complete the series of trials, while others had to be abandoned. The same was true in my experiment.

A number of *Cynomyia* responded to water on the first day of trials. Of the 32 flies tested, 5 responded occasionally, while 8 became consistently responsive to water. The cause of this condition was not ascertained. In every case but one, the animals had recovered on the following day, and remained unresponsive to water until the last 24 hours of life. At this time, following responsiveness to very low concentrations of saccharose, the flies again became responsive to water. Moreover, they drank at each water trial until immediately before death. The death of the animals usually took place from 3 to 8 hours after the water-responsive period began. Table IX shows a typical protocol. It will be noted that the threshold of this animal to saccharose fell rapidly on the sixth day after which the animal became water-responsive.

The behavior of the four species to water during the period of trials with saccharose solutions may be summarized as follows: *Lucilia* practically never became water-responsive. Certain individuals of *Calliphora erythrocephala*, *C. vomitoria*, and *Cynomyia* became water-responsive at one time or another, most commonly on the day before death. *Calliphora vomitoria* and *C. erythrocephala* gradually became water-responsive, responding more and more frequently to water as the trials went on. *Cynomyia*, on the contrary, became suddenly and consistently responsive.

In the course of trials, adventitious proboscis responses were occasionally encountered which varied with the species. In the case of *Lucilia* there was occasionally a tendency for the proboscis to be extended along the ventral side of the body, and to remain in that position more or less permanently. This presented a difficulty which frequently necessitated permanently abandoning the animal. Sometimes, also, as the animals were being taken from the rack to be tested, the proboscis sprang out. It was always immediately retracted, however, and remained inactive in the water trial. This response may have been due to a mechanical stimulus, but its cause could not be determined with certainty. *Lucilia* showed little irregularity of response, its responses being, with rare exceptions, regular and consistent.

Calliphora erythrocephala was quite the opposite of *Lucilia* with respect to the behavior described above. Short, casual extensions of the proboscis were not infrequently observed between trials. Sometimes these prevented the making of trials, since one could not be certain whether the animal was responding to the sugar solution or not. Several cases of more or less permanent proboscis extension were also encountered. A third difficulty was occasioned by the free leg coming in contact with the mouth parts and effecting their extension. Finally, the irregularity response was so great in this species that the threshold could not be determined in a number of cases.

Many of the difficulties with *Calliphora erythrocephala* occurred on the first day of trials. It seemed possible, therefore, that either the age when mounted or the tendency to regurgitate after the heavy feeding of the previous day might be responsible. Experiments were accordingly made to test these possibilities. From the same culture 10 animals 0–24 hours old and 10 animals 24–48 hours old were mounted and fed in the usual way. These two groups were carefully examined four times daily at intervals of 1 hour for slight proboscis movements and for permanent extensions of the proboscis. The response to water was also tested. The results were similar in the two groups, however. It was clear, therefore, that the age at time of mounting made no difference in the behavior in question.

Calliphora erythrocephala regurgitated considerably following feeding. To determine whether this was responsible for the behavior in question, 5 more flies, 0–24 hours old, from the same culture were mounted but not fed sugar solution. The results were much like those obtained with the group which was fed. Thus, while regurgitation may have been a factor, it could not have been entirely responsible for the results obtained.

In general, *C. vomitoria* was very consistent in its behavior. Irregularity of response was observed in only one case, animal No. 9, Table V. There were two cases of non-responsiveness to sugar solutions up to and including $\frac{64}{100}$ M on the first day of the trial (Table V). Apparently these flies were as yet too satiated to respond to this concentration. On the following day, however, they began to respond in the usual way.

Cynomyia was also a most favorable experimental animal. Only one individual exhibited casual proboscis movements, but this animal showed these consistently from the time of mounting until its death on the fifth day. There were a few cases of non-responsiveness to sugar solutions, the most pronounced case being animal No. 24, Table VII. This animal became non-responsive to saccharose on the fourth and fifth days. This was very unusual and is difficult to explain, since the animal was again responsive to saccharose on the sixth day.

Daily saccharose thresholds during a period of water diet were determined for all four species. In the case of *Lucilia*, 57 individuals were measured. The results obtained are illustrated by an experiment summarized in Table II and the protocol in Table III. It will be noted that the thresholds remained rather constant for the third and fourth days of water diet. The threshold then fell, but not very greatly, few of the flies responding to less than $\frac{1}{800}$ M. Moreover, the responses below this concentration were never consistent. The animals remained fairly constant at the lowest threshold reached for 1 or 2 days, after which they became moribund and consequently unfit for further trials. If the moribund period could have been shortened or eliminated, lower thresholds to sugar might have been obtained. The lowest threshold to saccharose recorded for *Lucilia* was between $\frac{1}{3,200}$ M and $\frac{1}{800}$ M.

The thresholds for *Calliphora erythrocephala* are illustrated by the results in Table IV. As previously noted, this species was more or less erratic in its response to saccharose. Some of the 37 animals measured showed high thresholds, while others showed rather low thresholds at the beginning of trials. There was a fairly sharp fall on the third day, while the fourth was again variable. The lowest threshold of response to saccharose for this species was between $\frac{1}{6,400}$ M and $\frac{1}{1,600}$ M; this was recorded in only one instance. In several cases, however, a threshold between $\frac{1}{3,200}$ M and $\frac{1}{1,600}$ was observed.

TABLE II

Showing the results of an experiment on *Lucilia sericata* in which the threshold of the right foreleg was determined daily, with the exception of 1 day, during the period of water diet. The concentrations are stated in terms of molarity. The following symbols are used to indicate conditions which at times prevented the determination of the threshold: *n*, non-responsive to concentrations up to and including $\frac{64}{100}$ M; *r*, lack of regularity in response; *w*, water responsive; the sign (?) after a concentration indicates that the response to this concentration was not quite uniform since there was a change from the general condition in one trial.

Animal No.	Period of Water Diet						
	Third Day, 48–52 Hrs.	Fourth Day, 71–74 Hrs.	Fifth Day, 95–98 Hrs.	Sixth Day, 119–22 Hrs.	Seventh Day, 143–46 Hrs.	Eighth Day, 170–74 Hrs.	Ninth Day, 194–98 Hrs.
25	1/400 – 1/100	*r*	1/800 – 1/200	1/800 – 1/200	Moribund	Dead	
26	4/100 – 8/100	2/100 – 8/100	1/100 – 4/100	1/400 – 2/100	Moribund	Dead	
27	*n*	2/100 – 16/100	1/100 – 4/100	1/800 – 1/100	Dead		
28	8/100 – 16/100	2/100 – 8/100	Moribund	Dead			
29	*w*	4/100 – 32/100	1/100 – 2/100	1/100 – 4/100	1/800 – 1/100	1/1,600 – 1/100	Dead
30	2/100 – 4/100	1/100 – 4/100	2/100 – 4/100	1/800 – 1/100	1/200 – 1/100	Dead	
31	1/1,600 – 1/200	1/100 – 16/100	1/800 – 1/200	Dead			
32	2/100 – 8/100	1/800 – 2/100	1/400 – 2/100	Dead			
33	2/100 – 8/100	1/100 – 2/100	1/400 – 1/200	1/800 – 1/100	Dead		
34	2/100 – 8/100	1/100 – 4/100	1/200 – 16/100	1/400 – 4/100	Dead		
35	2/100 – 4/100	2/100 – 4/100	1/100 – 8/100	1/100 – 4/100	1/800 – 1/400	1/200 – 1/100 ?	Dead
36	2/100 – 16/100	1/100 – 4/100	1/800 – 2/100	1/200 – 2/100	Dead		
37	8/100 – 16/100	2/100 – 8/100	1/100 – 16/100	1/800 – 1/400	1/200 – 1/100	1/800 – 4/100	Dead
38	1/100 – 4/100	1/1,600 – 1/100	*r*	1/800 – 1/100	Dead		
39	Weak	8/100 – 16/100	2/100 – 8/100	1/100 – 2/100	1/400 – 2/100	Dead	

TABLE III

Showing a typical protocol for *Lucilia*. Symbols: o, no response; 1, feeble response; + vigorous response.

Period of Water Diet	Molarities of Saccharose															Thresholds assigned
	1/25,600	1/12,800	1/6,400	1/3,200	1/1,600	1/800	1/400	1/200	1/100	2/100	4/100	8/100	16/100	32/100	64/100	
Third day, 46–49 hours										o o o o	1 + 1	+				$\frac{2}{100}$ M.–$\frac{4}{100}$ M.
Fourth day, 70–73 hours									o o o	1 o	+ + + +					$\frac{1}{100}$ M.–$\frac{4}{100}$ M.
Fifth day, 97–100 hours									o	o o o o	+ + + +					$\frac{2}{100}$ M.–$\frac{4}{100}$ M.
Sixth day, 115–18 hours						o o o	o o +	+ o	+ + +	+						$\frac{1}{800}$ M.–$\frac{1}{100}$ M.
Seventh day, 140–43 hours						o	o	o o	+ +							$\frac{1}{200}$ M.–$\frac{1}{100}$ M.
Eighth day, 164–68 hours	Dead															

TABLE IV

Showing the results of an experiment on *Calliphora erythrocephala* in which the threshold of the right foreleg was determined daily during the period of water diet. The concentrations are stated in terms of molarity. The following symbols are used to indicate conditions which at times prevented the determination of the threshold: *c*, casual movements of the proboscis; *p*, proboscis more or less permanently extended; *w*, water-responsive.

Animal No.	Period of Water Diet					
	Second Day, 23–27 Hrs.	Third Day, 52–56 Hrs.	Fourth Day, 74–78 Hrs.	Fifth Day, 98–102 Hrs.	Sixth Day, 124–28 Hrs.	Seventh Day, 146–48 Hrs.
1	2/100 – 8/100	4/100 – 8/100	4/100 – 16/100	1/100 – 4/100	1/1,600 – 1/400	Moribund; dead eighth day
2	1/3,200 – 1/400	1/200 – 2/100	1/100 – 2/100	*w*	Dead	
3	2/100 – 8/100	1/100 – 2/100	2/100 – 4/100	*w*	Dead	
4	8/100 – 16/100	1/800 – 1/100	*w*	Dead		
5	1/200 – 4/100	1/400 – 4/100	*w*	Dead		
6	*c*	2/100 – 4/100	1/200 – 8/100	Dead		
7	*w*	1/400 – 2/100	1/400 – 1/200	*w*	Moribund	Dead
8	16/100 – 64/100	2/100 – 8/100	1/100 – 8/100	*w*	Dead	
9	16/100 – 64/100	2/100 – 4/100	1/100 – 8/100	*w*	Dead	
10	4/100 – 64/100	1/100 – 8/100	1/100 – 8/100	*w*	Dead	
11	2/100 – 16/100	1/100 – 4/100	2/100 – 16/100	Dead		
12	2/100 – 8/100	1/200 – 2/100	1/800 – 1/200	Moribund	Dead	
13	*p*	*p*	*p*	Dead		
14	*w*	*w*	1/100 – 8/100	*w*	Dead	

The threshold data for 10 *C. vomitoria* are presented in Table V. This species showed a daily decline of responsiveness to saccharose during a period of water diet. The protocol in Table VI is typical.

TABLE V

Showing the results of an experiment on *Calliphora vomitoria* in which the threshold of the right foreleg was determined daily during the period of water diet. The concentrations are stated in terms of molarity. Symbols as in Table II.

Animal No.	Period of Water Diet						
	Second Day, 18–21 Hrs.	Third Day, 43–46 Hrs.	Fourth Day, 73–76 Hrs.	Fifth Day, 95–98 Hrs.	Sixth Day, 119–22 Hrs.	Seventh Day, 143–47 Hrs.	Eighth Day, 167 Hrs.
1....	2/100 – 4/100	1/400 – 4/100	1/12,800 – 1/3,200	1/6,400 – 1/1,600	Dead		
2....	1/100 – 4/100	1/800 – 2/100	1/200 – 8/100	1/1,600 – 1/100	Moribund	Dead	
3....	2/100 – 16/100	1/400 – 2/100	Dead				
4....	1/100 – 16/100 ?	1/200 – 8/100	1/400 – 1/100	1/3,200 – 1/400	1/12,800 – 1/3,200 *w*	Dead	
5....	2/100 – 8/100 *w*	1/800 – 2/100	1/400 – 2/100	1/6,400 – 1/1,600 *w*	Dead		
6....	*n*	1/200 – 16/100	1/100 – 4/100	1/400 – 2/100	1/6,400 – 1/1,600 *w*	*w*	Dead
7....	4/100 – 32/100	2/100 – 4/100	1/1,600 – 1/800	1/12,800 – 1/3,200 *w*	Dead		
8....	*n*	2/100 – 32/100	1/400 – 1/100 ?	1/200 – 2/100 *w*	Dead		
9....	*r*	1/100 – 2/100	*w*	Dead			
10....	*w*	Dead					

In this case, the threshold to saccharose showed a very slow decline on the second and third day of water diet, then a sudden drop on the fourth day. On the last day the animal became partially responsive to water. Taking the animals as a whole, responses were occasionally

TABLE VI

Showing a typical protocol of *Calliphora vomitoria*. Symbols: o, no response; 1, feeble response; +, vigorous response; *w*, indicates that the animal was partially water-responsive.

Period of Water Diet	Molarities of Saccharose															Thresholds Assigned
	1/25,600	1/12,800	1/6,400	1/3,200	1/1,600	1/800	1/400	1/200	1/100	2/100	4/100	8/100	16/100	32/100	64/100	
Second day, 18–21 hours											o o o o	+ o	o +	+ + 1 +		$\frac{4}{100}$ M.–$\frac{32}{100}$ M.
Third day, 43–46 hours									o	o o o o	+ + + +					$\frac{2}{100}$ M.–$\frac{4}{100}$ M.
Fourth day, 73–76 hours					o o o o	+ + + +	+		+		+					$\frac{1}{1,600}$ M.–$\frac{1}{800}$ M.
Fifth day, 95–98 hours		o o	+ + o	+	+						*w*					$\frac{1}{12,800}$ M.–$\frac{1}{3,200}$ M.
Sixth day, 119–22 hours	Dead															

TABLE VII

Showing the results of an experiment on *Cynomyia cadaverina* in which the threshold of the right foreleg was determined daily during the period of water diet. The concentrations are stated in terms of molarity. Symbols as in Tables II and IV.

Animal No.	Period of Water Diet						
	Second Day, 22–26 Hrs.	Third Day, 44–48 Hrs.	Fourth Day, 70–74 Hrs.	Fifth Day, 96–102 Hrs.	Sixth Day, 122–30 Hrs.	Seventh Day, 146–50 Hrs.	Eighth Day, 170–74 Hrs.
15	$\frac{2}{100}-\frac{8}{100}$	$\frac{2}{100}-\frac{8}{100}$	$\frac{4}{100}-\frac{8}{100}$	$\frac{1}{400}-\frac{1}{100}$	$\frac{1}{12,800}-\frac{1}{3,200}$	Dead	
16	*c*	*c*	*c*	Dead			
17	$\frac{1}{200}-\frac{2}{100}$	$\frac{2}{100}-\frac{8}{100}$	$\frac{2}{100}-\frac{8}{100}$	$\frac{1}{800}-\frac{1}{100}$?	$\frac{1}{6,400}-\frac{1}{800}$	Dead	
18	$\frac{8}{100}-\frac{16}{100}$	$\frac{2}{100}-\frac{4}{100}$	$\frac{1}{200}-\frac{4}{100}$	$\frac{1}{6,400}-\frac{1}{3,200}$	Dead		
19	$\frac{4}{100}-\frac{8}{100}$	$\frac{1}{100}-\frac{8}{100}$	$\frac{2}{100}-\frac{16}{100}$	$\frac{1}{200}-\frac{2}{100}$	$\frac{1}{6,400}-\frac{1}{400}$	Dead	
20	$\frac{8}{100}-\frac{64}{100}$ *w*	$\frac{1}{200}-\frac{8}{100}$	$\frac{2}{100}-\frac{4}{100}$	$\frac{1}{200}-\frac{1}{100}$	$\frac{1}{1,600}-\frac{1}{800}$	Dead	
21	*w*	$\frac{2}{100}-\frac{8}{100}$	$\frac{1}{100}-\frac{2}{100}$	$\frac{1}{6,400}-\frac{1}{800}$	$\frac{1}{3,200}-\frac{1}{1,600}$	$\frac{1}{6,400}-\frac{1}{1,600}$ *w*	Dead
22	$\frac{1}{200}-\frac{1}{100}$ *w*	$\frac{1}{200}-\frac{32}{100}$	$\frac{1}{100}-\frac{8}{100}$	$\frac{1}{800}-\frac{2}{100}$	$\frac{1}{3,200}-\frac{1}{1,600}$ *w*	Dead	
23	*n*	$\frac{2}{100}-\frac{4}{100}$	$\frac{4}{100}-\frac{32}{100}$	$\frac{2}{100}-\frac{16}{100}$	$\frac{1}{200}$?$-\frac{1}{100}$	Dead	
24	$\frac{4}{100}-\frac{32}{100}$ *w*	$\frac{2}{100}-\frac{32}{100}$	*n*	*n*	$\frac{1}{1,600}-\frac{2}{100}$	Dead	
25	$\frac{1}{200}-\frac{4}{100}$ *w*	$\frac{4}{100}-\frac{8}{100}$	$\frac{1}{200}-\frac{4}{100}$	$\frac{1}{3,200}-\frac{2}{100}$	Dead		
26	$\frac{8}{100}-\frac{32}{100}$	$\frac{4}{100}-\frac{8}{100}$	$\frac{1}{100}$?$-\frac{2}{100}$	Dead			

TABLE VIII

Showing a protocol of *Cynomyia cadaverina*. Symbols: o, no response; 1, feeble response; +, vigorous response.

Period of Water Diet	Molarities of Saccharose															Thresholds Assigned
	1/25,600	1/12,800	1/6,400	1/3,200	1/1,600	1/800	1/400	1/200	1/100	2/100	4/100	8/100	16/100	32/100	64/100	
Second day, 22–26 hours									o	o o o	+ o	+ + +				2/100 M.–8/100 M.
Third day, 44–48 hours										o o o o	+ o	+ + +				2/100 M.–8/100 M.
Fourth day, 70–74 hours											o o o	+ + o	+ o	+ + +		4/100 M.–32/100 M.
Fifth day, 96–102 hours								o	o o	+ + o	+ o	+ + +				1/100 M.–8/100 M.
Sixth day, 122–30 hours								o o o o	o + + o	+ o + o	o 1	+ + +				1/200 M.–8/100 M.
Seventh day, 146–50 hours	Dead															

TABLE IX

Showing a typical protocol of *Cynomyia cadaverina*. Symbols: o, no response; 1, feeble response; +, vigorous response. A concentration followed by (?) indicates doubt as to interpretation given.

Period of Water Diet	Molarities of Saccharose														Thresholds Assigned
	1/25,600	1/12,800	1/6,400	1/3,200	1/1,600	1/800	1/400	1/200	1/100	2/100	4/100	8/100	16/100	32/100	64/100	
Second day, 22–26 hours	Water-responsive															
Third day, 44–48 hours										o	o o o	1 1 + +				$\frac{4}{100}$ M.–$\frac{8}{100}$ M.
Fourth day, 70–74 hours											o o o	+ o	+ + +			$\frac{4}{100}$ M.–$\frac{16}{100}$ M.
Fifth day, 96–100 hours										o o o	o +	1 + o	+ + +			$\frac{2}{100}$ M.–$\frac{16}{100}$ M.
Sixth day: 122–25 hours						o o o 1	+ + o	+ + + +		1						$\frac{1}{800}$? M.–$\frac{1}{200}$ M.
125–28 hours			o	o o +	+ + +											$\frac{1}{3,200}$? M.–$\frac{1}{1,600}$ M.
128–30 hours	Water-responsive															
Seventh day, 146–50 hours	Dead															

obtained to $\frac{1}{6,400}$ M, but $\frac{1}{3,200}$ M was the lowest concentration to cause consistent response in any individual. There were no responses

TABLE X

Showing the average upper limit of the threshold to saccharose for four species of *Calliphoridae* during a period of water diet. The concentrations are expressed in terms of molarity. The data secured for *Calliphora erythrocephala* on the fifth and sixth days and for *Cynomyia* on the seventh day are not very significant, owing to the small number of cases. The averages for *Calliphora vomitoria* include the data of Minnich (1929) in addition to my own.

Species		Period of Water Diet					
		Second Day 20–24 Hours	Third Day 52–56 Hours	Fourth Day 74–76 Hours	Fifth Day 96–102 Hours	Sixth Day 120–25 Hours	Seventh Day 144–48 Hours
Lucilia sericata	Number of individuals averaged	21	38	47	30	17	6
	Threshold to saccharose	.124	.116	.068	.052	.03	.015
Calliphora vomitoria	Number of individuals averaged	9	13	11	14	8	7
	Threshold to saccharose	.124	.077	.025	.012	.0006	.0004
Cynomyia cadaverina	Number of individuals averaged	21	28	27	25	17	1
	Threshold to saccharose	.179	.122	.08	.026	.009	.0006
Calliphora erythrocephala	Number of individuals averaged	20	22	22	2	1	
	Threshold to saccharose	.248	.079	.07	.04	.003	

to $\frac{1}{12,800}$ M. It was, however, not always possible to determine the threshold on the last day of life because of water-responsiveness. In general, the lowest threshold to saccharose for *C. vomitoria* was between $\frac{1}{12,800}$ M and $\frac{1}{3,200}$ M. These results confirm those of Minnich on the same species.

For *Cynomyia*, saccharose thresholds were determined for 32 individuals. A summary of one experiment is given in Table VII. This species was much like *Calliphora vomitoria*. A consistent fall of the threshold, however, was not without exception. Thus, Table VIII shows a protocol in which the saccharose threshold failed to fall. This was not characteristic of the species as a whole. In general there was a gradual decline in the threshold of response to saccharose until the morning of the fifth or sixth day, when the threshold literally plunged down to a final or near-final level. Occasionally a further fall could be detected in the afternoon of the same day. This is illustrated in the protocol in Table IX. Three of the 32 animals became consistently responsive to $\frac{1}{6,400}$ M saccharose. There was an occasional response to $\frac{1}{12,800}$ M, but none to $\frac{1}{25,600}$ M. The lowest threshold of response was thus between $\frac{1}{25,600}$ M and $\frac{1}{6,400}$ M.

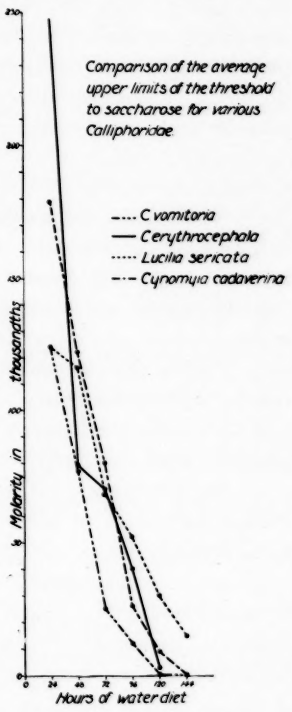
Comparison of the average upper limits of the threshold to saccharose for various Calliphoridae
C vomitoria
C erythrocephala
Lucilia sericata
Cynomyia cadaverina
Molarity in thousandths
Hours of water diet

FIG. 1.—Graphs based on the data given in Table X, showing the fall of saccharose threshold during a period of water diet in four species of Calliphoridae. The average upper limits of the threshold for each species are plotted against time. For simplicity, the time is expressed in terms of even 24-hour intervals, instead of the exact hours given in the table.

It is possible that critical tests with saccharose might be made on *Cynomyia* during the period of water-responsiveness by testing an animal immediately after it has drunk water following a water trial. This was not tried out. However, if the tests had been made, lower thresholds to sugar solutions might have been obtained.

The daily average upper limits of the threshold to saccharose for all the species tested are shown in Table X. These data are plotted in Figure 1. Both the table and the graphs show very clearly the downward trend of the threshold to saccharose as the water-diet period progresses. This is true of all four species. Furthermore, the graphs for *Cynomyia* and *Calliphora vomitoria* are much alike in the manner and extent of the fall.

V. GENERAL SUMMARY AND CONCLUSIONS

1. A series of experiments, based on the technique developed by Minnich, is described for *Lucilia sericata*, *Calliphora vomitoria*, *C. erythrocephala*, and *Cynomyia cadaverina*.

2. On an exclusive water diet *Lucilia* lived 6–8 days; *Calliphora erythrocephala*, 4–6 days; and *C. vomitoria* and *Cynomyia*, 5–7 days.

3. During the period of water diet *Lucilia* practically never became "water-responsive." *Calliphora erythrocephala*, *C. vomitoria*, and *Cynomyia* occasionally became water-responsive at one time or another, most commonly on the day before death.

4. The four species of flies readily distinguished water from saccharose solutions of appropriate concentration, by means of the right foreleg.

5. As the period of water diet progressed, the threshold of response to saccharose fell in all four species.

6. The threshold of response to saccharose in *Lucilia* was much higher on the last 2 days of water diet than that of the other three species, the lowest threshold of response being between $\frac{1}{3,200}$ and $\frac{1}{800}$ M. Only four instances of response were recorded to concentrations below $\frac{1}{1,600}$ M—viz., two to $\frac{1}{6,400}$ M and two to $\frac{1}{3,200}$ M.

7. *Calliphora erythrocephala* was most erratic in its responses to saccharose. This was not due to its tendency to regurgitate or to its age when mounted. Its lowest threshold of response, found in only 1 animal, was between $\frac{1}{6,400}$ M and $\frac{1}{1,600}$ M. Several individuals, however, reached a threshold between $\frac{1}{3,200}$ M and $\frac{1}{1,600}$ M.

8. The lowest saccharose threshold for *C. vomitoria* was between $\frac{1}{12,800}$ and $\frac{1}{3,200}$ M, which confirms the results of Minnich for this species.

9. The lowest threshold of response to saccharose for *Cynomyia* was between $\frac{1}{25,600}$ M and $\frac{1}{6,400}$ M.

10. The way in which the threshold for saccharose falls in the four species of Calliphoridae corresponds with that obtained by Minnich for the nymphalid butterfly *Pyrameis atalanta*, and the blowfly *Calliphora vomitoria*.

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PLACENTAL PERMEABILITY TO INSULIN IN THE ALBINO RAT

(Two figures)

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IT HAS been reported by Snyder and Hoskins (1927) that the placenta of the rabbit is not permeable to insulin from fetus to mother. Likewise these workers (1928) failed to obtain positive results with parathyroid extract. They found, nevertheless (1928), that glucose may pass readily from fetus to mother or in the opposite direction. Carlson and Drennan (1911) observed that diabetic dogs may be protected by the fetuses *in utero* in the latter part of pregnancy. Using goats, Pack and Barber (1930) found that insulin passed readily through the placenta from fetus to mother, although the number of their experimental animals was limited to four. Naeslund (1928) makes the observation that glucose may pass from the mother to the fetuses *in utero*, but states that the passage of glucose through the placenta produces a much smaller rise in blood sugar in the fetus than in the mother. Schlossman (1930) also reports similar results.

These findings indicate the desirability of a thorough study of placental permeability to insulin from fetus to mother and in the reverse direction in a graded series of fetuses, in order that the age of the fetuses may be taken into consideration in relation to the blood-sugar content of the mother. Three series of experiments were deemed necessary: (*a*) the determination of the sugar content of the blood of normal fetuses, (*b*) the determination of the extent of placental permeability to insulin from mother to fetus, and (*c*) the determination of the extent of placental permeability to insulin from fetus to mother.

THE SUGAR CONTENT OF THE BLOOD IN NORMAL FETUSES

The method of blood-sugar determination employed was that of Folin (1928). It was found impossible to obtain 0.10 cc. of blood

from the younger fetuses; therefore 0.05 cc. of blood was drawn and precipitated in one-half the usual volume of dilute tungstic-acid solution. The remainder of the procedure was carried out as for 0.10 cc. of blood. This method precluded making the determinations in duplicate, but the large number of determinations which were made offset this disability. In order to check the accuracy of the method, two samples were drawn from each adult control: one of 0.10 cc. and the other of 0.05 cc. The results checked within the experimental error.

The technique used in drawing the blood samples was rapid and simple. A pregnant rat was sufficiently stunned by a blow in the head to render it unconscious. The animal remained insentient throughout the procedure, but breathed regularly and deeply. Laparotomy was performed; and the fetuses, as needed, were quickly removed from the uterus with the membranes intact. Hemorrhage on separation of the placenta from the uterine wall seldom exceeded one drop of blood. As soon as one fetus was removed from the uterus, the amniotic sac was slit, the crown-rump length and sex recorded, and the blood sample drawn. Nicholas (1928) points out the necessity of holding the placenta above the fetus while drawing blood samples, because of the tendency of the blood of the fetus to run toward the placenta. This practice was found necessary and adopted. Both jugular veins were severed at a single cut by a pair of fine scissors, and the sample drawn into a calibrated pipette. Approximately 5 minutes elapsed from the time that the mother was stunned until all samples were secured and the proteins precipitated in the tungstate solution. All fetuses were viable, as evidenced by spontaneous movement and attempts at respiration. A maternal blood sample was taken from the heart immediately after fetal samples had been drawn. One hundred and eight fetuses, comprising 16 litters, were used. Twenty-four adults were used as controls, seventeen of these being females and seven males.

Table I presents the data obtained. The number of fetuses used in each litter, the average crown-rump length, the average blood-sugar content in milligrams per cent for each litter, and the variation in each litter are listed. The maternal blood-sugar values are also included.

In Figure 1 is represented the blood-sugar content for the various lengths of fetuses employed. The maternal blood-sugar value for each litter has been superimposed for comparison. As noted in a previous paper (1930), the stages of development are represented in terms of crown-rump length.

The 13 mm. stage was found to be the earliest at which adequate blood samples could be obtained. Even then, it was necessary to

TABLE I

MATERNAL AND FETAL BLOOD-SUGAR VALUES DURING THE LATTER THIRD OF THE GESTATION PERIOD

Number of litters used, 16.

Number of fetuses used, 108; males, 47; females, 57; undetermined, 4.

Average fetal blood sugar, 69 mg. per cent. Average maternal blood sugar, 112 mg. per cent.

Litter No.	No. of Fetuses Used	Average Crown-Rump Length (Mm.)	Range of Fetal Blood Sugars	Average of Fetal Blood Sugars	Maternal Blood Sugar
14*	12	13.0	57–64	60	85
8†	10	16.4	52–87	62	99
11	6	18.0	55–76	64	79
12	4	22.2	59–98	64	103
16	6	22.7	60–73	64	90
7	7	23.0	58–92	67	111
13	7	23.8	69–83	74	111
1	7	24.1	49–74	60	83
15	7	24.7	46–50	47	77
3	7	29.1	51–67	57	121
10	7	29.4	44–66	57	141
2	6	32.0	57–63	60	144
9	7	34.2	72–105	83	154
4	7	35.3	73–105	91	167
5	4	38.2	90–103	96	125
6	4	40.7	91–100	96	99

* It was necessary to use the blood of four fetuses for each determination.

† Blood from two fetuses used for each determination. In subsequent experiments one fetus was used for each determination. All of the fetuses in a litter were seldom used.

use four fetuses to secure sufficient blood for each determination. At the 16.4 mm. stage two fetuses were employed for each test. The blood sugar at the 13 mm. stage averaged 60 mg. per cent. This value rose rather gradually until a fetal length of 23.8 mm. was reached. Here a precipitate drop in the blood sugar occurred, followed by a subsequent gradual recovery up to the 32 mm. length, when the blood-sugar value was 60. The rise now became well defined until a value of 96 mg. per cent at 38.2 mm. was attained.

From this point onward the blood sugar was maintained at a practically uniform level until parturition.

Snyder and Hoskins (1928) have published somewhat similar results, employing the rabbit. They state that from the twenty-second to the thirty-second day of gestation the blood-sugar value rises from 33 to 100 mg. per cent, approaching the maternal blood-sugar

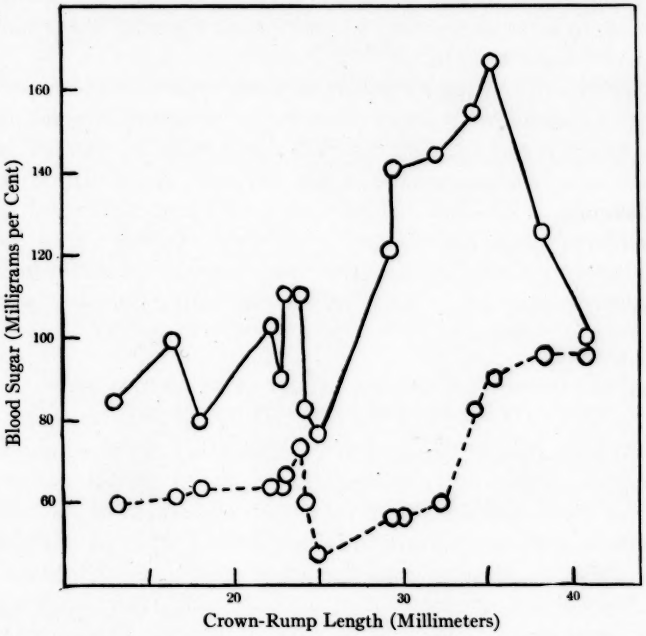

FIG. 1.—Showing maternal (solid line) and fetal (dash line) blood-sugar values during the latter third of the gestation period.

level at birth. Late in pregnancy in the cat it has also been shown that the fetal blood sugar is lower than that of the mother (Britton, 1930).

When the present fetal values are compared with those for the maternal blood, a general correlation is at once evident. The maternal blood sugar rises and falls, for the most part, with that of the fetuses *in utero;* a variation being present, however, from a fetal

length of 35.3 mm. until birth. At this portion of the curve the fetal blood sugar is seen to maintain a rather constant level, whereas the maternal blood-sugar value diminishes until it closely approximates that of the fetus at the time of parturition. No explanation of this phenomenon appears evident. It is suggested that the visceral and skeletal muscular contractions preparatory to parturition, and therefore the (increased) utilization of the carbohydrate stores, may be responsible in some degree for this precipitate lowering of the maternal blood-sugar content.

The average blood-sugar content of adult control rats was observed to be significantly lower than that of pregnant animals. In a series of seven males the average was found to be 86 mg., and in seventeen non-pregnant females 89 mg. per cent. No sexual difference, therefore, is apparent. These figures are comparable with the average of 112 mg. per cent found in the series of sixteen pregnant animals, but in no case did any of the control animals attain the high blood-sugar value found in the pregnant rats during the latter part of the gestation period.

THE DETERMINATION OF THE EXTENT OF PLACENTAL PERMEABILITY TO INSULIN FROM MOTHER TO FETUS

The same method of blood-sugar determination described above was used throughout these experiments. Pregnant rats were selected and initial blood samples drawn from the caudal vein of the tail. The mother was then injected intraperitoneally with 10 units of insulin. This dosage never caused marked symptoms of hypoglycemia. The animals remained inactive when undisturbed; but when handled, they reacted similarly to normal animals. This appears worthy of remark, since a considerable fall in the blood sugar occurred in many cases (53 and 55 per cent in animals No. 1 and No. 3, respectively). (See Table II.) The fetuses were in all cases viable, as evidenced by normal spontaneous movements.

One hour after insulin injection the animal was stunned by a blow on the head and a second maternal blood sample was obtained from the jugular vein. Fetal blood samples were drawn as in the case of the control series, and the crown-rump length and sex of each fetus recorded. The maternal and fetal blood-sugar values were then de-

termined. The difference between the first and second maternal blood-sugar values yielded the percentage fall in the mother. By comparison between the control and experimental fetuses, the percentage fall in the fetal blood sugar was determined for each litter. In the latter calculation the blood-sugar content of the control litter most nearly approximating the experimental litter in crown-rump length was taken as the normal. Table II shows the result of these experiments.

TABLE II

EFFECT UPON THE FETAL BLOOD SUGAR OF INSULIN INJECTION IN THE MOTHER

Number of litters used, 12.
Number of fetuses used, 71; males, 31; females, 40.
Average fetal blood sugar following insulin injection in the mother, 48 mg. per cent.

Litter No.	Initial Maternal Blood Sugar	Second Maternal Blood Sugar	Fall (per Cent)	No. of Fetuses Used	Average Crown-Rump Length (Mm.)	Range of Fetal Blood Sugar	Average of Fetal Blood Sugar	Fall in Blood Sugar as Compared with Controls (per Cent)
9*	103	84	18	8	16.8	44–46	45	27
8*	129	66	49	7	18.3	45–51	47	36
12*	100	54	46	8	18.4	46–50	48	33
5	87	50	43	8	22.2	42–48	46	28
6	120	97	19	7	24.1	36–44	40	22
2	108	64	41	4	29.2	30–39	34	36
10	81	43	47	6	30.1	35–36	36	35
4	103	63	38	5	30.8	49–55	53	12
3	98	45	55	5	34.4	56–59	56	33
7	120	97	19	5	37.2	51–56	53	44
11	128	62	44	6	41.0	56–67	64	34
1	93	43	53	2	41.8	55–57	56	42

* It was necessary to use the blood of more than one fetus for each determination. In all other cases the blood of one fetus was used in each determination. All of the fetuses in a litter were seldom used.

The litter number, initial maternal blood-sugar content, the second maternal blood-sugar value, and the percentage fall in the maternal blood sugar are included. The number of fetuses in each litter, the average crown-rump length of the litter, the range of individual determinations within the litters, the average fetal blood-sugar value for each litter, and the fall in blood-sugar content as compared with the control litter most nearly approximating the experimental litter in crown-rump length, constitute the data tabulated.

Examination of this table reveals the fact that in three cases (Nos. 9, 6, and 7) the fall in the maternal blood sugar was only 18–19 per cent. These cases appear to be exceptional, since the range in the remainder of the series was from 38 to 55 per cent.

The average fetal blood-sugar value one hour following insulin injection in the mother was 48 mg. per cent as compared with 69 mg. per cent in the control series (see Table I).

The percentage fall in the fetal blood sugar ranges from 12 to 44 per cent, the great majority being between 25 and 35 per cent.

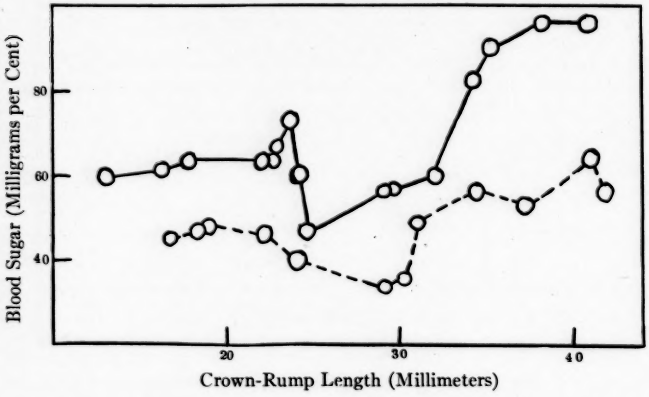

FIG. 2.—Curve showing blood-sugar values in fetuses following insulin injection in the mother (dash line) compared with normal fetuses (solid line).

The average initial maternal blood-sugar content averaged 105 mg. per cent, compared to 112 mg. per cent for the control series.

Figure 2 presents a graphic comparison between the blood-sugar content of the normal fetuses and those in which the mother was injected with insulin. The fluctuations in the curve of the experimental fetuses approximate those of the control curve throughout the first half, being at all times at a much lower level. At 23–24 mm. crown-rump length, both curves show a decline in the blood-sugar content. At approximately 30 mm. both series show a rapid rise in the blood-sugar level. From this point onward to term the curve of the normal fetuses continues to rise, while that of the animals in-

fluenced by the insulin maintains a more constant level. This may possibly be due to an increased activity of the islet tissue of the fetus at this age, an increased reaction to insulin, or an increased placental permeability to insulin. In no instance does the blood-sugar level of the animals treated with insulin even closely approximate that of normal animals of the same crown-rump length.

THE PERMEABILITY OF THE PLACENTA TO INSULIN FROM FETUS TO MOTHER

The same method of blood-sugar determination was used in this series of experiments. The procedure employed follows. The mother was anesthetized with amytal (80 mgs. per kilogram of body weight) by intraperitoneal injection. One hour from the time that the animal was completely under the influence of the anesthetic the initial maternal blood sample was obtained from the vessels of the tail. Laparotomy was then performed, and one horn of the uterus was removed from the body cavity and placed upon the laparotomy sheet. Ten units of insulin were then distributed among the fetuses by intraperitoneal injection, the hypodermic needle (28 gauge) piercing the uterine wall, the amniotic sac, and the abdominal wall of the fetus. The technique for the intraperitoneal injection of the rat fetus had been perfected and is referred to in an earlier work (Corey, 1930). The uterine horn was then replaced within the abdominal cavity of the mother, and the incision closed by means of two rows of continuous sutures. One hour after the injection of the fetuses with insulin the second maternal blood sample was drawn, the crown-rump length of the fetuses *in utero* recorded, and the animal then killed. During the foregoing procedure the body temperature of the mother was maintained by employing a water-jacketed operating table and several electric lamps. The blood-sugar content of the first and second samples was then determined. In every case an appreciable fall in the maternal blood sugar was found to occur. This fall was calculated in percentage of the initial blood-sugar level. In all animals thus treated (Nos. 1, 2, 3, and 4, Table IV) a positive result was obtained as regards the passage of insulin from fetus to mother through the placenta.

The possibility that amytal injection might conceivably influence

placental permeability to insulin was considered. Anderson, Chen, and Leake (1930) have reported that amytal produces no appreciable effect on the blood pressure of man. No data relative to the effect of this drug on the placenta has been found. However, the remaining animals of this series were treated in the following manner. An initial blood sample was obtained from the vessels of the tail. The animal was then placed under light ether anesthesia, and laparotomy and injection of the fetuses *in utero* performed as above. The wound was quickly repaired, and the animal returned to its cage. When the observed effects of the anesthetic had disappeared, usually in 10–20 minutes, the time was recorded. One hour after this the animal was stunned by a blow on the head and the second blood sample obtained from the jugular vein. The crown-rump length of the fetuses *in utero* was recorded and the mother then killed. The blood-sugar values were then determined. In every experiment of this series, a marked fall in the maternal blood sugar was observed. The results were therefore comparable to those obtained with amytal anesthesia.

Further control of the action of amytal anesthesia was deemed advisable. Six male control animals were treated in the following manner. After one hour's anesthetization with amytal an initial blood sample was obtained. The animal was allowed to remain under the anesthetic for another hour, and a second blood sample was then drawn and the animal killed. The blood-sugar content of the first and second samples was determined. No significant fluctuation in the values, which might be attributed to the effect of amytal injection, was found to occur.

Table III shows the result of these control experiments. It will be noted that in one case (animal No. 4) there is a considerable change in the blood-sugar level. Since this change is in the nature of an increase, it does not vitiate the experimental results. The remaining control animals show only such fluctuations in the sugar value as fall within the experimental error. It is therefore apparent that amytal does not affect significantly the blood-sugar level of the albino rat.

Two non-pregnant females were treated in the same manner as the experimental animals. Anesthetization with amytal, laparot-

omy, manipulation of the viscera, and repair of the wound were performed. No significant change in the glycemic level resulted. It is concluded that the amount of operative shock entailed by the experimental procedure does not tend to vitiate the results.

In order to ascertain whether the dosage employed affected the results, a series of animals was treated with graded doses of insulin. Two animals were given intraperitoneal injections of 5 units, two others 10 units, and eight additional animals were injected with 20 units. The average percentage fall in the blood sugar for the first

TABLE III

CONTROL ANIMALS USED TO DETERMINE THE EFFECT OF AMYTAL AND VISCERAL MANIPULATION UPON THE BLOOD-SUGAR LEVEL

No.	Sex	Treatment	Initial Sample (Blood Sugar)	Second Sample (Blood Sugar)
1	Male	Amytal injection	108	108
2	Male	Amytal injection	120	118
3	Male	Amytal injection	102	106
4	Male	Amytal injection	99	120
5	Male	Amytal injection	120	122
6	Male	Amytal injection	145	147
7	Female	Amytal injection, laparotomy, and repair	129	131
8	Female	Amytal injection, laparotomy, and repair	132	130

group was 31; for the second, 40; and for the third group, 51. In addition, seven male rats were injected intraperitoneally with 10 units of insulin, and four others with 20 units. They were observed over a period of 24 hours. At no time were symptoms of hypoglycemia apparent, and the animals were observed to eat and drink normally throughout this period. It is concluded that the dosage employed, while intentionally large, was not sufficient to produce insulin shock and was less than the maximum amount that could be tolerated by the animals. Voegtlin, Dunn, and Thompson (1924) state that the toxicity of insulin to the albino rat is least when given intraperitoneally, and greatest when administered subcutaneously. This may account in a measure for the tolerance to massive dosages exhibited by the animals used in this study.

Table IV shows the effect on the maternal blood sugar of insulin

injection into the fetuses. The crown-rump length of the fetuses *in utero*, their number, the initial maternal blood-sugar value, the maternal glycemic level one hour following the injection of the fetuses, and the fall in the maternal blood sugar expressed in percentage of the initial value are tabulated. In all cases a marked fall in the glycemic values occurred. This reduction varies from 14 per cent in rat No. 11 to 42 per cent in rats No. 4 and No. 10; the average being 28 per cent. The average initial level for this series was

TABLE IV

EFFECT UPON THE MOTHER OF INSULIN INJECTION IN THE FETUSES *in Utero*

Number of litters used, 12.
Number of fetuses used, 80.
Average initial blood sugar, 109.
Average blood sugar 1 hour after injection of fetuses, 79.
Average percentage fall in blood sugar, 28.

Litter No.	Crown-Rump Length (Mm.)	No. of Fetuses *in Utero*	Initial Maternal Blood Sugar (Mg. per Cent)	Maternal Blood Sugar 1 hour Later (Mg. per Cent)	Percentage Fall in Blood Sugar
1	13.0	5	117	97	17
11	14.6	6	136	117	14
6	26.2	10	97	69	29
5	26.4	7	98	62	36
4	27.1	7	132	77	42
7	28.3	7	100	60	40
8	28.7	7	83	64	22
9	29.1	7	139	100	28
12	31.8	8	102	75	26
2	33.3	7	87	67	23
10	39.5	4	107	62	42
3	41.2	5	112	93	17

109 mg. per cent as compared with 112 and 105 mg. per cent in the previous control and experimental series. These figures give an average of 109 mg. per cent for the blood-sugar value of the pregnant albino rat.

SUMMARY

The blood-sugar content in a series of normal fetal albino rats of different ages has been determined and compared with the maternal blood sugar. In the youngest fetuses that could be used for making determinations, those at the 13.0 mm. crown-rump stage, the values were found to be much lower than those of the maternal blood, i.e.,

60 mg. compared to 85 mg. per cent. Noteworthy differences in fetal blood sugar are observed at subsequent age periods. A decided rise occurs from the 32 mm. stage onward. At term the fetal value approximates that of the mother.

A correlation between the levels of the fetal and maternal blood sugar is evident throughout the series.

The average blood-sugar content of the pregnant albino rat is higher than that of non-pregnant animals. No significant differences were apparent, however, in non-pregnant female and male control animals.

The placenta of the albino rat is highly permeable to insulin from mother to fetus throughout the latter third of the gestation period. From the 32 mm. stage until term the placental permeability to insulin is greatly increased. That the fetus at this stage of development may be influenced by increased activity of the pancreatic islet tissue may be considered as a possibility.

During the latter third of the gestation period the placenta of the white rat appears to transmit insulin readily from fetus to mother. The greatest degree of permeability is observed when the fetuses are at the 27–29 mm. and the 39.5 mm. crown-rump stages. At term the placental permeability is reduced to a level similar to that when the fetuses *in utero* are at the 13.0 mm. stage.

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PHYSIOLOGICAL INTERRELATIONSHIP IN THE EYES OF DECAPOD CRUSTACEA

(One plate and three figures)

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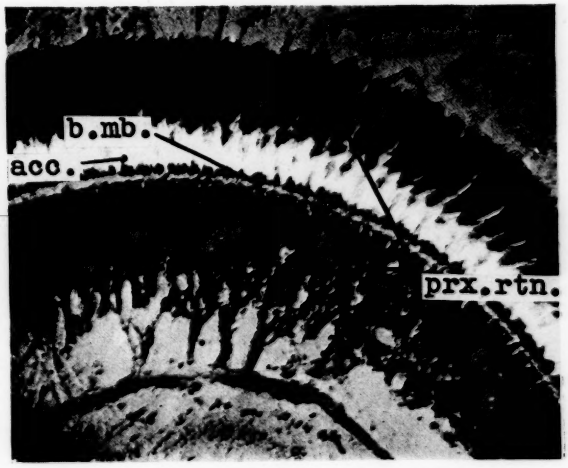
IT IS well known that in the proximal pigment cells (Fig. 1, *prx. rtn.;* and Plate I, *prx. rtn.*) of the ommatidia composing the compound eyes of crustaceans there is a cytoplasmic streaming which carries a dark pigment distally in the light and proximally in darkness. There is also movement of the distal pigment cells (Fig. 1, *dst. rtn.*) surrounding the cones (Fig. 1, *cn.*) and within the accessory pigment cells (Fig. 1, *acc.;* Plate, *acc.*) which form a reflecting layer. The last is poorly marked and is probably of minor importance. The first two responses have been generally regarded since the time of Exner (1891) as more or less adaptive, making available to the photoreceptors (the rhabdomes, Fig. 1, *rhb.;* Plate I, *rhb.*) a lesser or greater amount of light under conditions of light and darkness, respectively.

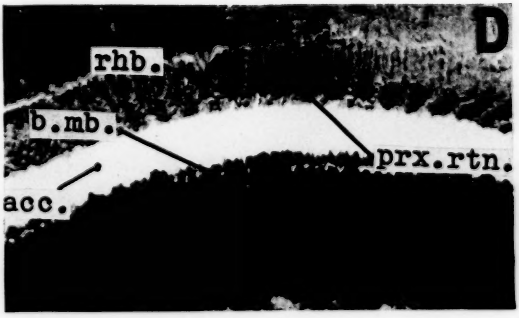
The existence of an interrelationship between the eyes of decapod crustaceans, whereby stimulation of one may under certain conditions produce a discernible effect on the position of the retinal pigment in the other, has been discussed by several investigators (Parker, 1897; von Frisch, 1908; Bennitt, 1924; Castle, 1927). Stated in another way, the question at issue is the independence of the retinal pigment cells. Light and darkness are, of course, the primary stimuli evoking pigmentary responses in the arthropod retina, and there is evidence that in some cases these responses are independent of all but the external stimuli, i.e, that the cells are at times true "independent effectors" (Parker, 1897; Castle, 1927). There is, however, a good deal of evidence that several secondary factors may also be present. Congdon (1907), for example, found that low temperature led to distal movement of the proximal retinal pigment in *Cambarus* and *Palaemonetes;* high temperature, to proximal movement. Demoll (1911) and Bennitt (1924) have noticed

PLATE I

Photographs of unstained sections of the compound eyes of *Cambarus virilis*, in the light-adapted (*L*) and dark-adapted (*D*) conditions. Only a part of each retina is shown, and only the proximal components of the ommatidia (cf. Fig. 1), viz.: *acc.*, accessory pigment layer; *b. mb.*, basement membrane; *prx. rtn.*, proximal retinal pigment cells, showing the nuclei at their distal ends, and extending proximally through the basement membrane; *rhb.*, rhabdomes, visible in (*D*) as the crenulated areas among the proximal retinal cells, but obscured in (*L*) by the retinal pigment. ×125.

PLATE I





that the "light-adapted" state always accompanies anesthesia or death. Bennitt and Merrick (1932) have obtained evidence that oxygen deficiency is followed by distal migration in the proximal pigment cells. Welsh (1930*a*) and Bennitt (1932) have observed rhythmical activity in the distal and proximal retinal pigment cells, respectively, which occurs even in the absence of the stimuli normally promoting these changes.

It is, of course, quite possible in most cases that the cellular reactions evoked by these "secondary" stimuli may have been due to the direct influence of the physical and chemical agents concerned on the pigmented cells themselves, quite apart from the effect of these agents on general metabolism. To that extent the retinal pigment cells might still be considered independent effectors. But it is hard to visualize a mechanism which brings about rhythmical activity in the absence of the usual stimuli, which is not in some way associated with the metabolic state of the animal; and if such association is present, there must be some means

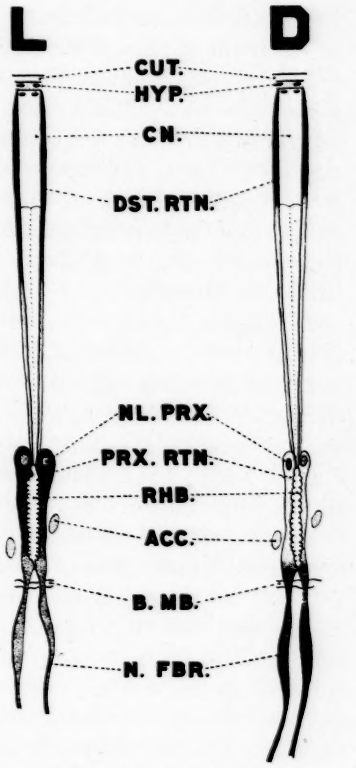


FIG. 1. Longitudinal sections of two ommatidia of *Cambarus virilis* Hagen, showing the arrangement of retinal pigment in the conditions characteristic for light (*L*) and darkness (*D*). Only two of the seven proximal retinal pigment cells are shown in each ommatidium. Abbreviations: *acc.*, nuclei of accessory pigment cells; *b. mb.*, basement membrane; *cn.*, cone; *cut.*, cuticle; *dst. rtn.*, distal retinal pigment cells; *hyp.*, cells of the hypodermis; *n. fbr.*, prolongations of the proximal retinal cells which form nerve-fibers into the optic ganglia; *nl. prx.*, nuclei of proximal retinal pigment cells; *prx. rtn.*, proximal retinal pigment cells; *rhb.*, rhabdome. ×125.

—nervous or vascular or both—whereby activity in the retina may be the result of physicochemical changes elsewhere in the body.

Parker (1897) concluded that the two retinas (of *Palaemonetes*) were independent of each other. This was based on the results of experiments in which one eye was covered while the other was left exposed to light; the exposed eyes "always showed an approach, more or less incomplete, to the condition characteristic for the dark" (p. 290). Further, when four dark-adapted eyes, excised with their optic stalks, were left for 2 hours in the light, three showed partial distal migration and the fourth did not change; when four excised light-adapted stalks were left for 2 hours in darkness, three showed partial proximal migration while the fourth had undergone complete proximal migration. Since there had been one case of complete proximal migration, he took this to indicate that the brain was not essential to the photomechanical changes in the retina. Finally, having cut off four dark-adapted retinas from their stalks and optic ganglia, leaving them then in the light for 2 hours, he found that all four showed a partial change, while in the case of four light-adapted retinas similarly isolated and left in the dark for 2 hours, two showed no change and two showed a partial change. Parker admitted the possibility of error through the passage of light through the translucent body of the animal, and of a slight influence from the optic ganglia, but believed, nevertheless, that each retinal cell was comparable "to a more or less independent organism, which receives a direct stimulus from the exterior, and responds appropriately" (1897, p. 295).

This also was the view of Castle (1927), who worked with the proximal retinal pigment cells of *Palaemonetes*. When both eyes were covered with an opaque mixture of plaster of Paris and lampblack, only half the eyes (thirteen) became fully dark-adapted; the rest were in various stages intermediate between the light- and dark-adapted conditions. It seems possible that this might have been ascribed to penetration of light through the animal's body, though it was not suggested by Castle. When one eye was covered and the other exposed, seven out of sixteen covered eyes became fully dark-adapted, the other nine being intermediate. From the fact that in several cases dark-adaptation had actually occurred in one eye while the other eye remained exposed, he concluded that

no "sympathetic" nervous interrelation of the eyes of *Palaemonetes* existed.

Von Frisch (1908) had obtained somewhat different results with the shrimp *Palaemon*. When one eye of a dark-adapted animal was covered, the proximal retinal pigment of the covered eye attained the proximal position, but that in the exposed eye moved only partially toward the distal position. Further, covering of one eye of a light-adapted animal resulted in only a partial migration of the pigment in both eyes. He also recognized that some of his results might have been due to diffusion of light through the body of the animal.

The writer (1924) used the crayfish *Cambarus*, whose size and opacity would presumably prevent stimulation of the eyes by any light save that impinging on them directly. After preliminary experiments to make sure that covering the eyes was tantamount to putting the entire animal in darkness, it was found that whenever one eye of a dark-adapted animal was covered and the animal left in the light for an hour, the proximal pigment in the covered eye showed an intermediate condition. When one eye of a light-adapted animal was covered and the animal left in the light, the proximal pigment in the covered eye did not go to the usual proximal position characteristic for darkness, but again remained intermediate. Some sort of a physiological relationship between the eyes was therefore thought to exist. It was the writer's view at that time that this was essentially a nervous relationship; the evidence now at hand, however, indicating that the circulation may be of even greater significance in relation to photomechanical changes in the crustacean retina, makes it desirable to withhold definite statement as to this point.

In the light of apparently conflicting results, it seemed best to reinvestigate the matter, using both *Palaemonetes* and as many large, opaque animals as were available. Observations were restricted to the photomechanical changes in the proximal retinal pigment cells.

MATERIALS AND GENERAL METHODS

The following animals were used: the crayfishes *Cambarus virilis* Hagen and *C. clarkii* Girard; the spider crab *Libinia emarginata* Leach; the rock crab *Cancer borealis* Stimpson; the green crab

Carcinides maenas (Linn.); the lobster *Homarus americanus* Milne-Edwards; the marine shrimp *Palaemonetes vulgaris* (Say); and the fresh-water shrimp *P. exilipes* Stimpson.

The animals were killed in hot water to fix quickly the position of the pigment, which was observed in one of two ways: (1) after sectioning in paraffin, following removal of the cuticle in the larger forms; (2) after maceration for 12–18 hours (depending on the size of the eye) in Bela Haller's fluid, followed by microscopic examination of the dissociated proximal pigment cells, to which the rhabdomes and accessory pigment cells almost always adhered. The two methods proved to be equally reliable, and the second was, of course, the simpler. Slides were made when especially important material made a permanent record desirable.

The experiments were performed by the writer and two graduate students, Miss M. Helen Thompson and Mr. F. E. Anspaugh—in part at the University of Missouri, in part at the Marine Biological Laboratory, Woods Hole, Massachusetts, and in part at the Franz Theodore Stone Laboratory of the Ohio State University, at Put-in-Bay, Ohio. Our thanks are due those in charge of the two biological stations for the facilities put at our disposal.

EXPERIMENTS

PALAEMONETES VULGARIS AND P. EXILIPES

The animals were fastened with U-shaped pieces of wire to a paraffin surface in a Petri dish, in such a way that respiratory movements were not impeded, while the eyes and top of the head projected above the water which flowed across the dish. One or both eyes were then covered with the opaque mixture of plaster of Paris and lampblack first used by Castle (1927), and the animals were left for 2 hours in the light. Parker (1897) had found 45 minutes sufficient for distal migration in the proximal pigment cells of *Palaemonetes* and 1 hour for proximal migration. Controls were placed in the dark, some fastened and others swimming free in the container.

The results of these experiments appear in Table I. Specific records of the positions reached by the proximal pigment were always kept, but for convenience in tabulation three general positions only

will be used: (1) "dark"—pigment not distal to the bases of the rhabdomes (Plate I, *D*); (2) "intermediate"—pigment extending along the rhabdomes but not distal to them; (3) "light"—pigment extending beyond the distal ends of the rhabdomes, i.e., almost or quite to the distal ends of the proximal retinal pigment cells (Plate I, *L*).

The normal, dark-adapted condition of the controls, both free and fastened, showed that enough time had been allowed for proximal migration and that fastening had no specific effect. The "intermediate" or "light" condition of all but one eye, in animals both of

TABLE I

EFFECT OF COVERING ONE OR BOTH EYES OF *Palaemonetes vulgaris* (*P. vul.*) AND *P. exilipes* (*P. ex.*) WITH AN OPAQUE MIXTURE OF PLASTER OF PARIS AND LAMPBLACK

		No. of Eyes					
		Dark		Intermediate		Light	
		P. vul.	*P. ex.*	*P. vul.*	*P. ex.*	*P. vul.*	*P. ex.*
Controls (in dark)	Free	49	18	0	0	0	0
	Fastened	43	*	0	*	0	*
Exposed (in light, fastened)	Both eyes covered	1	0	28	6	13	13
	One eye covered — Exposed eye	0	0	1	0	27	17
	One eye covered — Covered eye	0	0	9	1	19	12

* No controls were fastened in experiments on *P. exilipes*.

whose eyes had been covered, was probably due to passage of light through the body. When one eye was covered, the absence of any cases of complete proximal migration in the covered eyes, and the predominance of the "light" condition in the covered eyes, might be interpreted on the basis of influence from the other (exposed) eyes. It seems more reasonable to believe, however, that exposure of the other side of the head permitted more light to penetrate to the covered eye than when both sides of the head were covered. If these results indicate anything at all, in the writer's opinion, it is that such translucent animals as *Palaemon* and *Palaemonetes* are not suitable for a type of experiment in which the eye must be completely screened from outside light.

CAMBARUS, CANCER, CARCINIDES, LIBINIA, AND HOMARUS

Experiments with these larger crustaceans, against which the criticism of translucency cannot be made, took two forms: (1) one or both eyes were covered with plaster of Paris and lampblack and the animals were left in the light; (2) complete exclusion of light from all parts of the body except one eye, which was stimulated by light passing down a metal funnel cemented in place around the eye-stalk. Two modifications of this funnel apparatus were devised (Figs. 2 and 3).

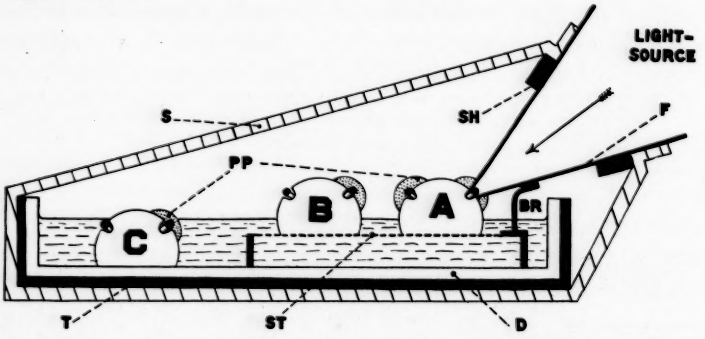


FIG. 2.—Funnel apparatus No. 1, shown in diagrammatic vertical section. A large crystallizing dish (*D*) is covered on the outside with a layer of heavy black tape (*T*). Crayfishes *A* and *B* are tied to a stage (*ST*) of galvanized iron screening in such a way that respiration is not impeded, while crayfish *C* moves freely in the water. One eye of crayfish *A* is cemented into a metal funnel (*F*) with plaster of Paris and lampblack (*PP*); the funnel is held in position by a metal brace (*BR*), and light is admitted to this eye through the funnel; the other eye of crayfish *A* is also covered with plaster of Paris and lampblack. One eye of the tied control, crayfish *B*, is covered, the other uncovered; the same is true of the free control, crayfish *C*. The whole apparatus is enveloped in an opaque covering consisting of four thicknesses of heavy black sateen (*S*) which is bound tightly around the funnel over a shoulder (*SH*). The interior of the apparatus was tested with photographic film for leakage of light.

Effect of covering only the eyes.—One eye of each of several light-adapted animals was covered, and the animals were left in the light for 6 hours. In the converse experiment, controls were first killed after 6 hours in darkness; with other animals which had been in darkness for the same period the entire anterior part of the head, including both eyes, was covered, the animal brought into the light, and the covering removed from one eye; exposure to light for 6 hours

followed. The results of these experiments appear in Table II. In this and subsequent tables the same three levels (dark, intermediate, and light) are used; the arrangement of parts of the ommatidia are in general the same. *Libinia* and *Cambarus* were the only genera found to show a noticeable degree of individual variation.

In one case (*Cambarus*), proximal migration proceeded to completion in the covered eye while the other remained exposed to light. This also occurred later in two individuals of *Libinia* (see

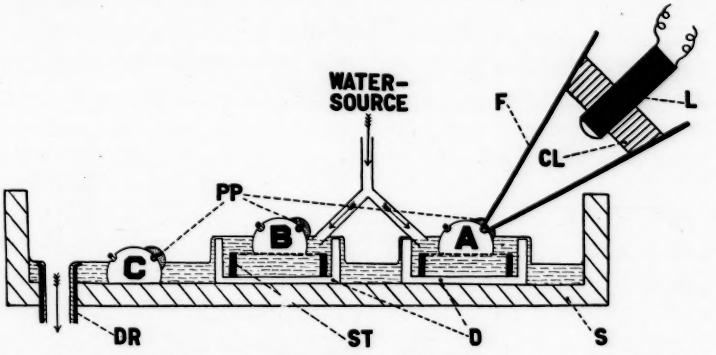


FIG. 3.—Funnel apparatus No. 2, shown in diagrammatic vertical section. Water flows through two crystallizing dishes (*D*) into a stone sink (*S*) and down the drain (*DR*). Each dish contains a crayfish (*A* and *B*) tied to a stage (*ST*) of galvanized iron screening. One eye of crayfish *A* is cemented into a metal funnel (*F*) with plaster of Paris and lampblack (*PP*); its other eye is uncovered. The tied control, crayfish *B*, has one eye covered, the other uncovered; the same is true of the free control, crayfish *C*, which moves freely in the sink. Light passes down the funnel from a flash-light (*L*) connected to dry cells and held in place by a plug of modeling clay (*CL*) about 2 inches thick, which also prevents the escape of light into the room. To measure the temperature within the funnel, a thermometer projected through the clay to the vicinity of the eye. The whole apparatus was located in a photographic dark-room carefully tested for the presence of small amounts of light.

Table III). If these were not examples of the considerable individual variation noticeable in these genera, and if one is justified in making a general statement on the basis of three such cases, then Parker (1897) and Castle (1927) were correct in their conclusion that independent proximal migration of pigment was possible. The other cases in which this did not occur still required explanation, as they apparently indicated an effect produced by stimulation of the

exposed eye. However, there was the possibility that some light might have leaked around the edges of the plaster case, or that in some way stimulation of the outside of the body by light might have accounted for the observed situation; the apparatus shown in Figures 1 and 2 was accordingly devised to eliminate error from these sources.

Effect of excluding light from all parts of the body except one eye.—As stated above, this was done by admitting light to the animal only

TABLE II

EFFECT OF COVERING ONE EYE WITH AN OPAQUE MIXTURE OF PLASTER OF PARIS AND LAMPBLACK

			No. of Eyes		
			Dark	Intermediate	Light
Light-adapted animals, left in the light	Exposed eyes (6 hours)	*Cambarus*	0	0	4
		Cancer	0	0	1
		Carcinides	0	0	2
		Libinia	0	0	1
	Covered eyes (6 hours)	*Cambarus*	1	0	3
		Cancer	0	0	1
		Carcinides	0	0	2
		Libinia	0	0	1
Dark-adapted animals, covered and brought into the light	Dark control (6 hours)	*Cancer*	2	0	0
		Libinia	4	0	0
	Exposed eyes (6 hours)	*Cancer*	0	0	1
		Libinia	0	0	2
	Covered eyes (6 hours)	*Cancer*	0	0	1
		Libinia	0	0	2

through a metal funnel cemented over the eye-stalk with plaster of Paris and lampblack. In apparatus No. 1 (Fig. 2) all the animals, including controls, were kept in a dish effectively screened from light by four thicknesses of heavy black sateen. In apparatus No. 2 (Fig. 3) the entire apparatus was kept in a photographic dark-room, the controls were allowed to move about freely in a sink of running water, and light (from an electric flashlight) was prevented from reaching them by sealing the flashlight within the funnel itself. In both cases, care was taken to test the surroundings of the animals

for leakage of light, by the use of photographic film, and in many of the experiments the unexposed eye of the animal was covered with plaster of Paris and lampblack as an added precaution. Further details in the construction of both pieces of apparatus are found in the legends accompanying Figures 2 and 3.

Early experiments of this sort showed that several factors other than light itself might possibly influence the position of the pigment, thus vitiating the results. These factors were identified and tested as follows:

1. Mechanical irritation caused by tying to the stage: Six crayfishes were tied to the metal stage used in these experiments, and six others were left free; the position of the retinal pigment was the same in all of them after 6 hours in darkness. Six crayfishes were tied to the metal stage and six more to glass finger bowls, with the same result.

2. Oxygen-deficiency due to overcrowding in the dishes: Some irregularity in the position of the proximal pigment, even exceeding the normal individual variation in the crayfish, was noticed in the earlier dark-controls when five or six animals were kept in the same dish. This led to the discovery that oxygen deficiency was associated with distal migration of the proximal pigment, even in darkness; this matter is discussed elsewhere by Bennitt and Merrick (1932). To guard against this, no more than three animals were kept in the same dish in experiments with apparatus No. 1, and in the experiments with apparatus No. 2 the animals were always in running water.

3. Local effect of the plaster cast: Neither Castle (1927) nor the writer had observed any such effect; but in order to detect its possible occurrence, one eye of each dark-control in these experiments was covered and the other left uncovered. No noticeable difference appeared in the covered and uncovered eyes of such controls.

4. Chemical effect of paint on the inside of the dish: This feature of the earlier experiments was eliminated by coating the outside of the dish with a layer of opaque black adhesive tape.

Table III shows the results of experiments on *Cambarus*, *Homarus*, and *Libinia*, using apparatus No. 1. There were two sorts of dark-control, one free and one tied to the stage; one eye of each was

covered. The unexposed eye of the remaining animal was covered. We were unable to think of any way in which light could get to the covered eye directly; consequently, any discernible effects within that eye were thought to be associated in some way with stimulation of the exposed eye by light.

With apparatus No. 2, *Cambarus* was the only animal used. One animal was killed in the light at the beginning of each experiment;

TABLE III

EFFECT OF EXPOSING ONLY ONE EYE OF *Cambarus*, *Homarus*, AND *Libinia* TO LIGHT, THE REST OF THE BODY BEING IN DARKNESS. APPARATUS NO. 1

			No. of Eyes		
			Dark	Intermediate	Light
Controls (in darkness)	Free; eyes uncovered	*Cambarus*	3	1	0
		Homarus	6	0	0
	Free; eyes covered	*Cambarus*	3	1	0
	Tied to stage; eyes uncovered	*Cambarus*	3	0	0
		Libinia	10	0	1
	Tied to stage; eyes covered	*Cambarus*	3	0	0
Exposed eye		*Cambarus*	0	0	4
		Homarus	0	0	6
		Libinia	1	2	8
Unexposed eye (covered)		*Cambarus*	0	0	4
		Homarus	0	2	4
		Libinia	2	2	7

a second was left free in the sink with one eye covered; a third was tied to the stage with one eye covered; the fourth was tied to the stage with the funnel over one eye, the other uncovered. The second, third, and fourth animals were left in this condition for 4 hours, when the light in the funnel was turned on. They were killed after 2 hours' exposure to light. Whereas the funnel in apparatus No. 1 was only about 5 inches long, the funnel in apparatus No. 2 was 9 inches long; this kept the light source about 5 inches from the eye, and the temperature within the funnel rose not over 3° C. during the ex-

posure, an amount insufficient to evoke an appreciable pigmentary response. The results of these experiments appear in Table IV.

DISCUSSION

After eliminating as many sources of error as we could, it nevertheless appeared that almost without exception the condition in the unexposed eye was unlike that in the various control eyes. If this is

TABLE IV

EFFECT OF EXPOSING ONLY ONE EYE OF *Cambarus*, THE REST OF THE BODY BEING IN DARKNESS. APPARATUS NO. 2

		No. of Eyes		
		Dark	Intermediate	Light
Controls	Killed in light	0	0	9
	Free, in darkness; eyes uncovered	9	0	0
	Free, in darkness; eyes covered	9	0	0
	Tied, in darkness; eyes uncovered	9	0	0
	Tied, in darkness; eyes covered	9	0	0
Exposed eyes		0	3	6
Unexposed eyes		0	9	0

correct, we must consider that internal changes, set up by stimulation of one eye in dark-adapted animals, constitute another "secondary" (and doubtless indirect) cause of pigment migration in the proximal retinal cells, along with temperature, oxygen-deficiency, anesthesia, and death.

There is still a good deal of doubt as to the nature of such internal changes. It was the view of Kiesel (1894) and Demoll (1911, 1917) with respect to insects, and of the writer (1924) with respect to Crustacea, that this form of stimulation "from within" was through the mediation of the nervous system. The main argument against this view is that, up to date, no efferent fibers have been found in the optic nerve of decapods, with the exception of eight or ten found by Parker (1895) in *Astacus*, which he believed to pass to the muscles

of the optic stalk. It is difficult to consider these responsible for a general response of the entire set of proximal pigment cells none of which has been shown to have any nervous connection other than that with afferent fibers into the optic ganglia.

On the other hand, the recent significant discovery of Perkins (1928) that the compound eyes of *Palaemonetes* were endocrine organs suggests a vascular mechanism which may perhaps be concerned with the observed interrelation between the eyes. The internal secretion of the eyes, always present, as shown by Perkins' injection experiments, but liberated into the circulation in larger amounts in response to stimulation by light from a white background, induced contraction of the skin chromatophores. This is especially noteworthy in view of the fact that retinal pigment cells are in many ways analogous to chromatophores in their action and in the agents by which they are influenced.

SUMMARY OF EXPERIMENTAL RESULTS

1. When both eyes of *Palaemonetes vulgaris* and *P. exilipes* were covered with plaster of Paris and lampblack and the animals left in the light, complete dark-adaptation of the proximal pigment cells occurred in only 1 out of 61 eyes, the rest being in a light-adapted or intermediate condition.

2. When one eye of *Palaemonetes* was covered, forty-four of the exposed eyes were light-adapted, one intermediate, and none dark-adapted; of the covered eyes, thirty-one were light-adapted, ten intermediate, and none dark-adapted.

3. Covering of one eye only, in four light-adapted individuals of *Cambarus*, one *Cancer*, two *Carcinides*, and one *Libinia*, resulted in dark-adaptation in one eye only (one individual of *Cambarus*); all the other covered eyes were light-adapted.

4. Covering of one eye only, in one dark-adapted *Cancer* and two *Libinia*, resulted in light-adaptation of both the exposed and the covered eyes.

5. After exposure of one eye to light, with the rest of the body in darkness following dark-adaptation, the four unexposed eyes of *Cambarus* were light-adapted; four unexposed eyes of *Homarus* were light-adapted and two were intermediate; seven unexposed eyes

of *Libinia* were light-adapted, two were intermediate, and two were dark-adapted.

6. With a different type of funnel-apparatus, six of the exposed eyes of *Cambarus* were light-adapted and three were intermediate; the nine unexposed eyes were intermediate.

7. Since in these experiments one individual of *Cambarus* and two individuals of *Libinia* showed apparently independent proximal pigment migration in the covered eye while the other eye remained exposed, the possibility of such independent action must be admitted, though these cases might be explained on the basis of the considerable individual variation noticed in *Cambarus* and *Libinia*. The very great majority of cases, however, gave evidence that distal migration in the unexposed, dark-adapted eye followed photic stimulation of the exposed eye.

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DIURNAL RHYTHM IN THE PROXIMAL PIGMENT CELLS OF THE CRAYFISH RETINA

(One figure)

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IN THE compound eyes of crayfish, shrimps, and other decapod Crustacea there are three sets of pigmentary effector-cells in which changes may be induced through the action of light and darkness: (1) the distal pigment cells, (2) the proximal pigment cells, and (3) the accessory pigment cells. Changes in the accessory pigment cells are not well defined, and little is known about them as yet. Welsh (1930*b*) has described the action of the distal pigment cells of *Palaemonetes* as a proximal movement in the light through the activity of contractile fibers, and a distal movement along the sides of the cone in darkness. The proximal pigment cells are of virtually fixed length and their cytoplasm in the light is filled with dark pigment, which in darkness is carried proximally to the vicinity of the basement membrane and below. The proximal and distal cells are evidently, at least in part, independent effectors. The extent to which they are under nervous influence is not yet known; but evidence is accumulating that the general metabolic condition of the animal, as manifest in chemical changes within the blood, may at times affect the state of the pigmented retinal cells (Demoll, 1911; Welsh, 1930*a;* Bennitt, 1924, 1932; Bennitt and Merrick, 1932). Perkins (1928) and Koller (1928) have already shown the effect of hormones produced by certain decapods on the "expansion" and "contraction" of their skin-chromatophores.

Some time ago, during experiments on pigment migration in the proximal retinal cells of the crayfish, the writer noticed that the pigment extended farther distally in dark-adapted animals killed during the day than in those killed during the night; all such animals had been kept in total darkness for 6 hours or more. This led to the idea that there might be here a diurnal rhythmic change, even in the absence of the stimuli normally provoking such change. Welsh

(1930*a*) has cited several cases of this sort among skin-chromatophores. Kiesel (1894) seems to have been the first to observe rhythmic activity in the arthropod retina; he kept two noctuid moths in total darkness for several weeks, and reported a daily variation in the position of the distal ("iris-") pigment, as evidenced by the phenomenon of "glow." Demoll (1911) observed similar changes in the moth *Agrotis pronuba*. Such happenings have been variously associated with degree of nervous influence, condition of health, metabolic periodicity, and the like.

Welsh (1930*a*) reported the first case of diurnal rhythm in the pigmented cells of the crustacean retina. When the shrimps *Macrobrachium olfersii* and *M. acanthurus* were kept constantly illuminated, their distal pigment cells migrated distally (i.e., to the position characteristic for darkness) about 6:00 P.M. and remained in this position until about 5:00 A.M., when they moved proximally again. He found no such change in the distal pigment cells of excised eyes, and concluded that "direct control of the distal pigment cells is by way of the blood, and that the persistence of diurnal rhythm under constant illumination parallels a periodicity in metabolism which is controlled by the nervous system" (p. 394).

In studying the proximal pigment cells of the crayfish retina, Welsh's method of direct observation could not be used. After killing in hot water to fix the position of the pigment, the eyes were left for 18 hours or more in Bela Haller's fluid. The separated proximal pigment cells, to which the rhabdomes and accessory pigment cells almost always adhered, were then examined microscopically. This method was unsatisfactory for the study of distal pigment cells, but comparison with mounted sections showed it to be a reliable means of determining the position of the proximal pigment with reference to such fixed levels as the basement membrane, rhabdomes, and tips of the proximal pigment cells.

Observations on the crayfish support Welsh's statement (1930*a*) that in *Macrobrachium* no discernible change in the position of the proximal pigment occurred when the animals were kept in the light. In all our experiments on the crayfish and other Crustacea it has been evident that the presence of light apparently supersedes all other influences in regulating the position of the proximal pigment.

Prolonged observations on excised dark-adapted eyes of the crayfish were not possible, since within a few hours the proximal pigment always assumed the position characteristic for light and remained there; Welsh noted that the distal pigment of *Macrobrachium* did the same thing. I have attributed this, in the crayfish at least, to death of the tissues, a condition invariably accompanied by migration of the proximal pigment to the "light" position (Demoll, 1911; Bennitt, 1924), and have therefore been unable to learn anything about the rôle of circulation and nervous influence in this rhythmic activity of the crayfish retina.

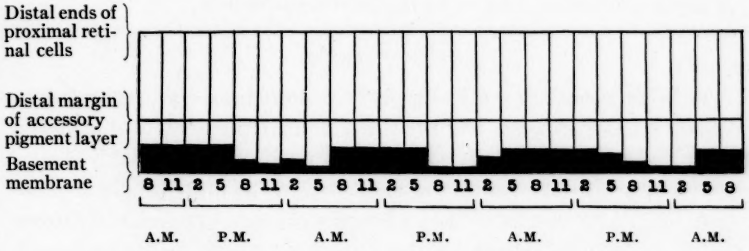
Distal ends of proximal retinal cells
Distal margin of accessory pigment layer
Basement membrane
8 11 2 5 8 11 2 5 8 11 2 5 8 11 2 5 8 11 2 5 8 11 2 5 8
A.M.
P.M.
A.M.
P.M.
A.M.
P.M.
A.M.

FIG. 1.—Extent of proximal retinal pigment of *Cambarus virilis*, taken at 3-hour intervals for 3 days following an initial 24-hour period of dark-adaptation. Animals in total darkness throughout the experiment. Numbers represent times at which eyes were fixed.

Since marked changes in temperature influence the position of the proximal pigment in both *Cambarus* and *Palaemonetes* (Congdon, 1907), temperature records were kept, which showed a variation of not over 1° C. for the duration of the experiment. The animals were alive and active when removed for killing.

To test for a periodic change while the animals (like those of Kiesel and Demoll) were kept in total darkness, seventy-five crayfish (*Cambarus virilis*) were first dark-adapted in running water for 24 hours. Three animals were then killed at the end of each 3-hour period for the next 75 hours; the eyes were macerated and examined, and the members of each trio were found to show identical pigment-positions in each instance.

In the accompanying figure, the position of the proximal pigment is shown with reference to three fixed levels: (1) the basement mem-

brane, (2) the distal margin of the accessory-pigment layer, and (3) the distal ends of the proximal pigment cells. In the fully dark-adapted state, the pigment extends but slightly distal to the basement membrane; this was found only for a period of 3–9 hours during the night. The fully light-adapted state was not found at all. The pigment-level during the day was intermediate between the two, and there was nothing like the sharp and extensive daily change that Welsh noticed in the distal retinal cells of *Macrobrachium* under constant illumination. However, the change was distinct and quite possibly might have been found in the proximal retinal cells of *Macrobrachium* by similar methods of observation.

CONCLUSIONS

1. With abundant water-supply, constant temperature, and continued total darkness, the proximal retinal pigment of *Cambarus* nevertheless shows a daily variation in position.

2. For 3–9 hours during the night it is in the most proximal position; for the remainder of the 24 hours it extends to a distinctly more distal position, though at no time does it approach the light-adapted position.

3. There appears to be a rough correlation between the position of the proximal pigment and the periods of light and darkness to which the animal is subjected in nature.

4. Such a change is most likely to be brought about by a metabolic periodicity in the animal, acting through the circulation.

5. Experiments with excised eyes have been unsuccessful.

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FOOD AS A SEX-DETERMINING FACTOR IN MOINA MACROCOPA

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THE amount of food available to crowded cladoceran mothers reared in manure infusions was dismissed as a causative factor in the production of males by such mothers by Banta and Brown (1929*a*, 1929*b*). They ascribe the production of males by crowded mothers to the accumulation of the animals' own excretory substances. Stuart and Banta (1929, 1931); Stuart, Tallman, and Cooper (1931); and Stuart, Cooper, and Tallman (1931), however, showed that under certain experimental conditions available bacteria as food were an important factor in the production of males by crowded mothers. According to past findings then, these two factors—excretory substances and food—have been suggested as being associated with sex control in Cladocera. When two factors appear to be associated with a single phenomenon, the question naturally arises as to whether either factor alone can, under any conditions, cause the results ascribed to both. Evidence now available on this point is inconclusive, we feel, since most findings may be interpreted as supporting the rôle of excretory substances alone, or the rôle of food alone, or possibly a composite effect of the two. Thus it becomes quite evident that entirely new methods of procedure must be devised before either factor can be completely ruled out in any one experiment. The following experiments were designed to exclude the possible intervention of excretory substances in the control of sex in the offspring of *Moina macrocopa* mothers.

PRODUCTION OF MALES BY ISOLATED MOINA MOTHERS REARED IN LARGE VOLUMES OF MEDIUM

Banta and Brown (1929*a*) found that a single *Moina* mother reared in 7.5 ml. of culture medium produced males. They construed this to mean that in such a small volume of medium a single mother was sufficiently influenced by her own excretory substances to pro-

duce males. It therefore occurred to the writers that if food, rather than excretory products, was a sex-controlling factor in these experiments, males should be produced by isolated mothers reared in amounts of medium far in excess of 7.5 ml., provided that the quantity of available food be sufficiently reduced by dilution.

One hundred milliliters of a Berkefeld V filtrate[1] of aged normal medium (manure infusion) were placed in each of five 4-oz. salt-mouth bottles. In a second series of five similar bottles were placed 20 ml. of the filtrate to which were added 80 ml. of pond water, paper filtered; while to a third series of five bottles were added 10 ml. of the filtrate together with 90 ml. of pond water. A single young sister-*Moina* was placed in each bottle; and as a brood control (to show that the mothers were not producing males irrespective of the environment) a single sister was placed in each of five bottles containing 25 ml. of normal medium. All bottles were incubated at 25° C., and when releases occurred, the young in each bottle were counted and sexed, and the number of eggs present in the brood chamber for the second release was noted. Six such series of experiments were run with the specified dilutions of filtrate, each series consisting of five separate experiments and the five brood controls of five bottles each. The results of these experiments are tabulated in Table I. The results of the five separate experiments in a single series have been totaled and tabulated under the series number. Thus, in Series 1 of the 5 mothers isolated in 100 ml. of the undiluted filtrate (1:1), only 3 mothers released. The total number of young and second eggs for these mothers will be found in the columns so marked. In dilution 1:5 all 5 mothers released a total of 27 young, of which 10 were males. In another column will be found the number of mothers in the series which produced males; of the 5 mothers in Series 1, dilution 1:5, 3 mothers produced males.

The results of these experiments were not very satisfactory in that there seemed to be little consistency in the appearance of males in the various dilutions. Likewise the total number of young released and the second eggs were so few that the animals were considered to be in a starvation medium. Moreover, the majority of broods were

[1] Berkefeld V filters are coarse candles which, while not removing all bacteria from a manure infusion, reduce the number considerably and remove all extraneous débris.

bisexual, a condition which we have recognized as being frequently associated with a stringent lack of food. It was, therefore, decided to use high dilutions of normal medium in filtered pond water rather

TABLE I

Series No.	100 Ml. of Normal Medium Filtrate																		Brood Controls 5 Isolated Mothers in 25 Ml. Normal Medium					
	Dilution 1:1						Dilution 1:5						Dilution 1:10											
	Mothers Releasing	Second Eggs	Total Young	Females	Males	Mothers Producing Males	Mothers Releasing	Second Eggs	Total Young	Females	Males	Mothers Producing Males	Mothers Releasing	Second Eggs	Total Young	Females	Males	Mothers Producing Males	Mothers Releasing	Second Eggs	Total Young	Females	Males	Mothers Producing Males
1	3	12	16	16	0	0	5	14	27	17	10	3	5	22	39	23	16	3	5	94	87	87	0	0
2	3	18	21	0	21	3	5	22	32	18	14	4	4	26	31	12	19	3	5	88	78	78	0	0
3	5	10	47	14	33	4	4	0	15	15	0	0	No releases						5	86	84	84	0	0
4	5	8	33	13	20	4	No releases						No releases						5	86	70	70	0	0
5	5	14	24	22	2	1	5	16	26	15	11	2	4	4	15	3	12	3	5	100	76	76	0	0
6	5	24	30	26	4	1	4	10	24	24	0	0	1	0	3	3	0	0	5	98	72	72	0	0

	100 Ml. of Normal Medium																		Brood Controls 5 Isolated Mothers in 25 Ml. Normal Medium					
	Dilution 1:75						Dilution 1:175						Dilution 1:500											
7	5	88	80	80	0	0	5	78	91	91	0	0	5	48	61	54	7	1	5	120	104	104	0	0
8	5	68	88	88	0	0	5	64	82	82	0	0	5	28	65	43	22	2	5	114	108	108	0	0
9	5	100	102	102	0	0	5	84	95	95	0	0	5	34	50	41	9	2	5	100	113	113	0	0
10	5	84	100	100	0	0	5	78	96	96	0	0	5	28	56	39	17	2	5	124	107	107	0	0
11	5	38	57	57	0	0	5	18	57	48	9	1	5	4	36	16	20	3	5	86	67	67	0	0
12	5	50	54	54	0	0	5	22	39	35	4	1	5	16	40	28	12	2	5	60	72	72	0	0
13	5	54	93	93	0	0	5	26	67	23	44	5	3	6	17	11	6	2	5	74	104	104	0	0
14	5	84	81	81	0	0	5	29	63	53	10	1	5	10	61	49	12	3	4	84	80	80	0	0
15	4	36	62	56	6	1	5	12	73	73	0	0	5	0	35	24	11	2	5	88	81	81	0	0
16	5	30	56	35	21	2	5	36	62	54	8	1	5	2	32	0	32	5	5	102	90	90	0	0
17	5	46	80	80	0	0	5	26	61	42	19	2	5	22	53	53	0	0	5	100	95	95	0	0
18	5	38	72	72	0	0	5	16	50	13	37	4	5	11	30	15	15	3	5	96	94	94	0	0
19	5	28	64	51	13	1	5	14	39	26	13	2	5	9	22	15	7	2	5	100	94	94	0	0
20	5	72	105	105	0	0	5	64	105	105	0	0	5	54	84	84	0	0	5	48	108	108	0	0

Total mothers released	95	93	82	99
Average second eggs per mother	9.4	6.7	3.9	18.6
Average young per mother	13.3	11.8	8.9	18.0
Percentage of males	9.4	16.2	29.7	0
Percentage of mothers producing males	17.8	27.9	46.3	0

TABLE I—*Continued*

Series No.	250 Ml. of Normal Medium																Brood Controls 5 Isolated Mothers in 25 Ml. Normal Medium					
	Dilution 1:125						Dilution 1:1250						Pond Water									
	Mothers Releasing	Second Eggs	Total Young	Females	Males	Mothers Producing Males	Mothers Releasing	Second Eggs	Total Young	Females	Males	Mothers Producing Males	Mothers Releasing	Second Eggs	Total Young	Females	Males	Mothers Producing Males	Mothers Releasing	Second Eggs	Total Young	Females	Males	Mothers Producing Males
1	5	74	103	103	0	0	5	56	32	32	0	0	5	50	22	17	5	2	5	106	104	104	0	0
2	5	80	74	74	0	0	5	48	38	25	13	2	4	4	17	3	14	4	5	120	95	95	0	0
3	5	48	78	78	0	0	5	12	33	27	6	3	5	13	33	33	0	0	5	86	84	84	0	0
4	5	70	72	72	0	0	5	58	38	32	6	1	5	52	19	0	19	5	5	88	75	75	0	0
5	5	38	70	70	0	0	5	42	15	12	3	1	5	40	15	1	14	4	5	116	97	97	0	0
6	5	56	77	77	0	0	5	41	24	12	12	4	5	44	18	5	13	5	5	106	101	101	0	0
7	5	60	57	57	0	0	5	46	29	16	13	3	5	22	13	4	9	2	5	112	96	96	0	0
8	5	74	57	57	0	0	5	30	32	32	0	0	5	12	26	19	7	3	5	104	97	97	0	0
9	5	66	23	17	6	2	5	22	13	13	0	0	5	9	20	17	3	1	5	80	21	21	0	0
10	5	46	18	18	0	0	5	16	14	7	7	2	5	14	14	14	0	0	5	90	20	20	0	0
Total mothers released	50						50						49						50					
Average second eggs per mother	12.2						7.4						5.3						20.1					
Average young per mother	12.5						5.3						4.0						15.8					
Percentage of males	.9						22.3						42.6						0					
Percentage of mothers producing males	4.0						32.0						53.0						0					

	500 Ml. of Normal Medium																Brood Controls 5 Isolated Mothers in 25 Ml. Normal Medium					
	Dilution 1:125						Dilution 1:1250						Pond Water									
1	5	78	102	102	0	0	5	56	47	30	17	2	5	56	65	65	0	0	5	118	87	87	0	0
2	5	80	104	104	0	0	5	52	32	12	20	4	5	60	30	2	28	5	5	144	87	87	0	0
3	5	52	87	87	0	0	5	12	36	35	1	1	5	10	26	22	4	2	5	92	84	84	0	0
4	5	58	66	66	0	0	5	46	24	16	8	2	5	38	26	9	17	3	5	126	112	112	0	0
5	5	68	79	79	0	0	5	36	33	31	2	1	3	32	18	18	0	0	5	122	108	108	0	0
Total mothers released	25						25						23						25					
Average second eggs per mother	13.4						8.0						8.5						24.0					
Average young per mother	17.5						6.8						7.1						19.1					
Percentage of males	0						27.9						29.6						0					
Percentage of mothers producing males	0						40.0						43.4						0					

than the dilutions of filtrate. Preliminary experiments showed that the dilutions listed in Table I under 100 ml. of normal medium would prove satisfactory for our purposes in that the percentage of males produced by isolated mothers in these dilutions was somewhat proportional to the dilution, though it should be borne in mind that in these experiments we are primarily interested in the production of males in the volume specified rather than in the actual percentage of males produced in the different dilutions of any one volume. For convenience, the results of the experiments with filtrate and normal dilutions in the 100 ml. volume experiments are totaled together despite the different environments.

It will be noted in Table I that 30 per cent of the mothers releasing in the various dilutions of the 100 ml. experiments produced males, the percentage of mothers producing males increasing as the dilution of the medium increased. Similar results were obtained from isolated mothers reared in 250 ml. volumes of 1:125 and 1:1250 dilutions of normal medium in pond water. In the 1:125 dilution but 4 per cent of the 50 mothers produced males; in the 1:1250 dilution 32 per cent of the 50 mothers released males; while in pond water over one-half (53.06 per cent) of the 49 releasing mothers produced males. Of the 149 mothers releasing in 250 ml. volume experiments, 29.53 per cent produced males. Similar results were again obtained from isolated mothers in 500 ml. of the same dilutions, except that the percentage of mothers producing males is not as consistent with the dilution as in the other volumes. There were 73 mothers that released in the 500 ml. experiments; and of these, 27.39 per cent produced males. The percentage of mothers producing males in the different volumes decreases as the volume of the medium increases, but it is questionable whether the decrease is significant in view of the decrease in the number of experiments as the volume employed increases. In these experiments, the mothers in pond water and high dilutions of the medium were often retarded 12 hours or more in releasing their young. Experiments in the various volumes of medium (100, 250, and 500 ml.) were not run concurrently; neither was it possible to use young from sister-mothers in the different volumes. Therefore, no attempt should be made to relate volume of medium per se with the results obtained in these experiments.

PRODUCTION OF MALES BY CROWDED MOINA MOTHERS FREQUENTLY TRANSFERRED TO FRESH MEDIUM

When 10 *Moina* mothers are reared in 60 ml. of normal medium and allowed to develop to the point of egg-laying, there is, according to some investigators, an accumulation of excretory products from the mothers which controls the sex of the young produced. If, then, 10 mothers are reared in 60 ml. of medium and the animals, previous to egg-laying, frequently changed to fresh medium, thus preventing the accumulation of the excretory substances, one should obtain, for the most part, only female young. Several preliminary experiments served to show that under such conditions only female young are produced. But that one is preventing the accumulation of excretory products is by no means the only explanation of these results, since it may be contended that in frequently changing the animals to fresh medium, one is also renewing the food supply. If this is true, then by using a dilution of medium which would not furnish adequate food for the normal development of the mothers, a similar experiment should give results quite different from those obtained with normal medium, even though the volume of the diluted medium and the number of times the mothers are changed to fresh medium remain the same. Since frequently changing the animals to fresh medium would prevent the accumulation of excretory substances, the production of males would occur in a medium where there can be no accumulated excretory products, but where a lack of food only could explain the production of males.

Seven to eight sister-*Moina* shortly after being released were placed in each of three small bottles containing 35 ml. of normal medium (dilution 1:12.5) and the bottles incubated at 25° C. for 8–12 hours. At the end of this time all the mothers in one bottle were transferred to another bottle containing the same amount of normal medium (noted as dilution 1:12.5 in Table III), the mothers in a second bottle were transferred to 35 ml. of a 1:50 dilution of normal medium, while the mothers in the third bottle were transferred to 35 ml. of a 1:150 dilution (all dilutions being made with pond water). Two hours later the mothers in normal medium were again transferred to a similar amount of normal medium, the mothers in the 1:50 dilution transferred to a fresh portion of 1:50 dilution, and

the mothers in the 1:150 dilution treated in the same manner. This procedure was repeated every 2 hours until eggs were laid. From nine to twelve transfers of the mothers in the various dilutions were required before the eggs were laid. As the mothers approached the critical period, great care was exercised in transferring the animals to avoid, as far as possible, agitating them. Furthermore, the mothers were not examined microscopically for the presence of eggs until a macroscopic examination made it fairly certain that the eggs had been laid. It was found unnecessary to use the microscope to determine the presence of eggs in the mothers reared in normal medium, but in the highest dilutions of medium the change in size and shape of the mothers at egg-laying was imperceptible to the eye. These precautions were taken because the highest possible percentage of males was desired in these experiments. (It will be recalled that Banta and Brown [1929*b*] have shown that aëration of crowded mothers on a glass slide before the critical period serves to decrease the male percentage produced by such mothers, and it will be shown later by us that mechanical agitation of the mothers exerts the same influence.) As soon as the mothers in any dilution had laid eggs, the mothers were transferred to a bottle containing 50 ml. of normal medium and allowed to release. When releases occurred, the number of mothers surviving, the number of eggs laid by the mothers for the second brood, the total number of young, and the sex of 100 young in so far as possible were determined. Thirteen series of experiments were run, each series consisting of from five to ten separate experiments. In any one series of experiments all the young used were the offspring of sister-mothers and all the medium employed throughout any one series of experiments came from the same batch. The results of these experiments will be found tabulated in Table II. The results of the separate experiments in the different series are totaled and tabulated under the series number.

It will be seen in Table II that, despite the frequent changes of the mothers to fresh medium, males have been abundantly produced in the higher dilutions of medium. The 813 mothers reared in the 1:12.5 dilution (normal medium) produced a total of 13,672 young, of which only 2.41 per cent were males. In the 1:50 dilution 793 mothers produced 11,704 young, of which 35.05 per cent were males;

TABLE II

Series No.	No. of Mothers per Bottle	No. of Separate Experiments per Series	No. of Transfers per Series	Dilutions of Concentrated Manure Infusions																											
				1:12.5									1:50								1:150										
				Surviving Mothers	Second Eggs	Total Young	Females	Males	Separate Experiments Producing Males	Average Young per Mother	Average Second Eggs per Mother	Percentage of Males	Surviving Mothers	Second Eggs	Total Young	Females	Males	Separate Experiments Producing Males	Average Young per Mother	Average Second Eggs per Mother	Percentage of Males	Surviving Mothers	Second Eggs	Total Young	Females	Males	Separate Experiments Producing Males	Average Young per Mother	Average Second Eggs per Mother	Percentage of Males
1.....	7	10	10	69	748	1,295	1,097	19	2	18.7	10.8	1.7	67	486	864	322	541	10	12.8	7.2	62.6	68	328	655	101	554	10	9.6	4.8	84.5
2.....	7	5	12	34	248	941	628	0	0	27.6	7.2	0	34	136	828	601	32	4	24.3	4.0	5.0	33	32	630	497	88	5	19.0	.9	15.0
3.....	7	5	12	35	420	232	232	0	0	6.6	12.0	0	34	528	271	266	5	1	7.9	15.5	1.8	35	350	257	151	106	5	7.3	10.0	41.2
4.....	7	10	10	65	842	722	683	39	4	11.1	12.9	5.4	66	852	796	541	255	10	12.0	12.9	32.0	64	664	456	378	78	9	7.1	10.3	17.1
5.....	7	6	12	41	520	429	424	5	2	10.4	12.6	1.1	40	442	371	281	90	6	9.2	11.0	24.2	35	250	173	104	69	6	4.9	7.1	39.8
6.....	7	10	11	69	428	1,223	1,040	69	4	17.7	6.2	6.2	62	272	759	517	242	10	12.2	4.3	31.8	68	612	450	73	377	10	6.6	9.0	83.7
7.....	7	10	9	69	720	1,076	1,004	52	5	15.5	10.4	4.9	65	520	773	447	326	9	11.8	8.0	42.1	65	506	576	446	130	8	8.8	7.7	22.5
8.....	7	10	12	65	1,090	968	968	0	0	14.8	16.7	0	63	864	953	863	90	6	15.1	13.7	9.4	65	852	742	610	132	10	11.4	13.1	17.7
9.....	8	10	12	75	1,222	1,506	1,256	19	3	20.0	16.2	1.4	75	1,042	1,259	965	211	9	16.7	13.8	17.9	72	998	1,132	616	497	10	15.7	13.8	44.6
10.....	8	10	11	77	370	1,500	1,268	13	2	19.4	4.8	1.0	75	98	1,364	456	874	10	18.1	1.3	65.7	79	176	1,181	132	1,049	10	14.9	2.2	88.8
11.....	8	10	12	78	406	1,441	1,196	0	0	18.4	5.2	0	75	211	1,361	667	502	9	18.1	2.8	42.9	78	102	918	210	708	10	11.7	1.3	77.1
12.....	7	10	9	68	816	1,030	919	58	5	15.1	12.0	5.9	68	556	1,038	565	438	9	15.2	8.1	43.6	66	348	628	348	280	10	9.5	5.2	44.5
13.....	7	10	10	68	722	1,309	1,194	21	1	19.2	10.6	1.7	69	503	1,067	759	308	9	15.4	7.2	28.8	70	434	819	283	536	10	11.7	6.2	65.4

	1:12.5	1:50	1:150
Total mothers.....	813	793	798
Average second eggs per mother.....	10.5	8.2	7.0
Average young per mother.....	16.8	14.7	10.7
Total young.....	13,672	11,704	8,617
Total females.....	11,909	7,250	3,949
Total males.....	295	3,914	4,604
Percentage of males....	2.4	35.0	53.8
Percentage of separate experiments producing males.....	24.1	87.9	97.4
Total experiments.....	116	116	116

whereas 798 mothers reared in the 1:150 dilution released 8,617 young, of which 53.82 per cent were males. It is true that some males have been produced in normal medium, but this seems to be almost inevitable in any experiment involving so large a number of mothers as this unless the mothers are reared in an extremely strong medium or highly bacterized filtrates. Of the 116 separate experiments in normal medium, males were produced in but 24.13 per cent of the experiments. In the more highly diluted medium (1:50), 87.90 per cent of the separate experiments produced males, while 97.40 per cent of the 116 experiments in the 1:50 dilution resulted in males. From these results it is quite evident that preventing the accumulation of excretory substances by changing the mothers to fresh medium at 2-hour intervals, starting 18–24 hours before egg-laying, has not prevented the production of males in dilute medium where there is an inadequate amount of food. Here again, as in Table I, the steadily decreasing number of young produced by the mothers in the various dilutions of medium is indicative of insufficient food. The average number of young per mother in the normal medium was 16.81; in the 1:50 dilution, 14.75; while in the 1:150 dilution, the average number of young per mother was but 10.79. It would seem rather conclusive, then, that the smaller number of young and the high percentage of males produced by the mothers in the more highly diluted medium in these experiments are the results of an insufficiency of food rather than of the accumulation of excretory products.

In these experiments it was noted that, as a rule, the mothers in normal medium (dilution 1:12.5) laid eggs before those in the other dilutions, while the mothers in the 1:50 dilution laid eggs before those in the 1:150 dilution in every series. There are, however, three notable exceptions which occurred in normal medium. The mothers in the 1:50 dilution in Series 3, 5, and 8 laid eggs from 2 to 4 hours before the mothers in the more concentrated medium (dilution 1:12.5). This is significant as it shows that a retardation in the mothers' development may occur even in normal medium, without subsequent production of males. Smith (1915) and Banta and Brown (1929*c*) concluded that male production in Cladocera is associated with or caused by a retardation in the mother's development pre-

vious to egg-laying. Stuart and Banta (1931) found that mothers reared in highly bacterized filtrates of normal medium were retarded in their releases more than sister-mothers reared in less highly bacterized filtrates, and stated that, as far as could be ascertained, the retardation occurred after the eggs had been laid in the brood chamber. This now appears to have been an error. Stuart, Tallman, and Cooper, in rearing mothers in varying dilutions of concentrated medium, came to the conclusion that in strong medium a retardation in the mothers' development before the egg-laying period could occur without subsequent production of males. The evidence was considered (in view of the previous statement by Stuart and Banta) insufficient to be emphasized in their paper. There appear to be, then, conditions under which *Moina* mothers may be retarded in their development before the egg-laying period *without the retardation being associated with or causing male production.* Further evidence of a similar nature will be presented in a later paper.

PRODUCTION OF MALES BY CROWDED MOINA MOTHERS IN FLOWING MEDIUM

In Experiment I isolated *Moina* mothers were reared in large volumes of medium, while in Experiment II crowded *Moina* mothers were reared in small volumes of medium which were constantly being renewed. It would seem that an ideal situation would be attained if the large volume factor of Experiment I could be combined with the constant renewal factor of Experiment II. To this end crowded mothers were reared in a corked glass tube of 80 ml. capacity through which paper or Berkefeld filtrates of normal medium were constantly flowing. (Since this method was soon abandoned for a better one, a detailed description of the apparatus is omitted.) This procedure served, however, to show that such an experiment was feasible. In three experiments conducted in this manner, 70 mothers produced 941 young, of which 21.56 per cent were males; appropriate controls of isolated sister-mothers in normal medium produced only female young. The rate of flow of the medium through the tube was such that each mother from early first instar to egg-laying was bathed in an average of 190 ml. of flowing medium. One objection to this experiment was that the mothers were being reared

in a closed system which, despite the flowing medium, might favor the retention of gaseous excretory products in the small amount of air entrapped in the tube. To obviate this objection the following modification of the procedure was made.

A 1-, 2-, or 3-liter flask filled with filtrate (hereafter called the "reservoir flask"), corked with a one-hole rubber stopper through which extended a piece of glass tubing 6×5/8 inches was inverted over a 4-oz. salt-mouth bottle containing 80 ml. of filtrate and 10 sister-mothers. As long as the glass tube from the reservoir flask is below the level of the liquid in the culture bottle, the system is static; but a siphon placed in the culture bottle immediately converts the system to a dynamic one, since as soon as the level in the culture bottle drops below the end of the tube from the reservoir flask, fresh medium will flow to the culture bottle, the rate of flow from flask to bottle being regulated by the rate of flow of the liquid from the culture bottle through the siphon. The action of this system is unique for our purposes in that it not only automatically serves to change the medium in the culture bottle but also serves mechanically to agitate the medium in the bottle, since the intermittent surges of filtrate from the reservoir flask cause rather turbulent swirls in the medium of the culture bottle. As a matter of fact, this latter feature is so pronounced as almost to prohibit the use of a 3-liter flask for a reservoir, because, when the flask is nearly empty, the force of the surges occasionally causes the culture bottle to overflow slightly, even though the normal level of the medium in the culture bottle is reduced to give a volume of 70 ml. Not only does the flowing medium militate against the accumulation of excretory substances, but mechanical agitation of the medium, in the manner described, further insures that there will be no retention of these substances (Banta and Brown, 1929*b*). It is important to note that the end of the glass tube from the reservoir flask which is immersed in the liquid of the culture bottle must be properly beveled, otherwise the system occasionally ceases to operate, especially when a conduit tube of smaller diameter than the one specified is used. Moreover, a piece of bolting cloth or other suitable material must be used to seal the end of the siphon in the culture bottle. In only two experiments did any of the Cladocera swim up the glass tube into the reservoir flask. All

experiments were carried out with the culture bottle and control bottle submerged to the level of the contained medium in a water bath which was maintained at approximately 25° C. The experiments were controlled by rearing 10 sister-mothers in 80 ml. of the filtrate (called "filtrate control") without irrigation, and also by rearing 5 isolated sister-mothers (called "brood control") in 50 ml. of normal medium. Nine experiments were done using paper or Berkefeld V filtrates of normal medium in the reservoir flask, but it was found with the latter that not infrequently a bacterial growth occurred sufficiently heavy to be detected with the eye,[1] which necessarily defeated the purpose of the experiment; such experiments were discarded as soon as the bacterial growth was noted. Several other experiments were also discarded because the filtration was so effective in reducing the food content of the medium that the mothers starved to death. Much better results on the whole were obtained with high dilutions of normal medium made with pond water. In either case, when the mothers in the filtrate control bottles laid eggs, the mothers in the irrigated and control bottles were transferred to bottles containing 80 ml. of normal medium and allowed to release. When releases occurred, the number of mothers surviving, the number of second eggs, the total number and sex of the young, were determined as previously described. Fifteen series of experiments were run, each series consisting of four separate experiments, the results of which will be found tabulated in Table III.

In the column marked "Character of Medium" symbols designating the type of medium used in the reservoir flask will be found: "P. F." meaning a triple, hard paper, filtrate of aged normal medium; "B. F." signifying a Berkefeld V filtrate of the same medium; while "N. M." 1:300 designates a 1:300 dilution of normal medium. In the next column is shown the amount of medium *per mother* that flowed through the irrigated bottles in the course of the experiment. The amount of medium per mother is by no means mathematically accurate, since in some experiments irrigation of the bottles continued for an hour or more after the eggs were laid. Moreover, the amount of medium per mother is computed on the basis of 10 moth-

[1] The Berkefeld filtration of a medium in which bacteria have been cultivated seems, on occasion, to remove substances inimical to the further growth of the bacteria.

TABLE III

Series Number	Separate Experiments per Series	Character of Medium Used*	Medium per Mother (in Ml.)	Irrigated Experiments									Unirrigated Experiments									Brood Controls—5 Isolated Mothers in Normal Medium								
				Surviving Mothers	Second Eggs	Total Young	Females	Males	Separate Experiments Producing Males	Average Young per Mother	Average Second Eggs per Mother	Percentage of Males	Surviving Mothers	Second Eggs	Total Young	Females	Males	Separate Experiments Producing Males	Average Young per Mother	Average Second Eggs per Mother	Percentage of Males	Surviving Mothers	Second Eggs	Total Young	Females	Males	Separate Experiments Producing Males	Average Young per Mother	Average Second Eggs per Mother	Percentage of Males
1.....	4	P.F.	200	39	....	417	266	151	4	10.6	....	36.2	37	....	354	121	233	4	9.5	....	65.8	....	....	....	....	....	....	....	....
2.....	4	P.F.	250	40	620	651	588	56	3	16.2	15.5	8.6	39	136	381	218	163	4	9.7	3.4	42.7	....	....	....	....	....	....	....	....
3.....	4	B.F.	300	22	12	227	138	89	4	10.3	.5	39.2	25	0	306	237	69	4	12.2	.0	22.5	....	....	....	....	....	....	....	....
4.....	4	B.F.	300	36	244	318	198	120	3	8.8	6.7	37.7	25	28	257	131	126	4	10.2	1.1	49.0	20	460	366	366	0	0	18.3	23.0	0
5.....	4	P.F.	250	38	130	402	247	120	3	10.5	3.4	32.6	39	60	209	18	191	4	5.3	1.5	91.3	20	420	336	326	10	1	16.8	21.0	2.9
6.....	4	P.F.	200	40	580	722	493	102	3	18.0	14.5	17.1	40	260	621	375	210	4	15.5	6.5	35.8	20	480	437	437	0	0	21.8	24.0	0
7.....	4	B.F.	325	40	640	556	431	108	4	13.9	16.0	20.0	38	250	259	95	164	4	6.8	6.5	63.3	20	500	396	396	0	0	19.8	25.0	0
8.....	4	P.F. N.M.	450	40	220	440	406	34	2	11.0	5.5	7.7	39	96	351	175	176	4	9.0	2.4	50.1	20	430	344	344	0	0	17.2	21.5	0
9.....	4	1:300 N.M.	500	39	680	565	329	134	4	14.4	17.4	28.9	40	420	49	49	0	0	1.2	10.5	0	20	440	392	392	0	0	19.6	22.0	0
10.....	4	1:300 N.M.	600	37	546	335	74	258	4	9.0	14.7	77.7	38	164	95	64	31	3	2.5	4.3	32.6	20	380	414	414	0	0	20.7	19.0	0
11.....	4	1:150 N.M.	600	39	680	471	363	108	4	12.0	17.4	22.9	40	420	409	177	232	4	10.2	10.5	56.7	20	640	595	595	0	0	29.7	32.0	0
12.....	4	1:125 N.M.	500	39	466	384	298	86	4	9.8	11.9	22.3	37	96	286	96	190	4	7.7	2.5	66.4	19	416	502	502	0	0	26.4	21.8	0
13.....	4	1:125 N.M.	650	40	640	558	363	58	3	13.9	16.0	13.7	39	408	345	144	201	4	8.8	10.4	58.2	20	640	495	495	0	0	24.7	32.0	0
14.....	4	1:125 N.M.	550	39	540	383	340	43	2	9.8	13.8	11.2	39	212	228	93	135	4	5.8	5.4	59.2	19	640	442	433	9	1	23.2	33.6	2.0
15.....	4	1:500	850	36	554	256	76	180	4	7.1	15.3	70.3	40	300	92	90	2	1	2.3	7.5	2.1	20	410	403	403	0	0	20.1	20.5	0

	Irrigated Experiments	Unirrigated Experiments	Brood Controls—5 Isolated Mothers in Normal Medium
Total mothers	564	555	238
Average second eggs per mother	11.6	5.1	24.6
Average young per mother	11.8	7.6	21.5
Total young	6,685	4,242	5,122
Total females	4,610	2,083	5,103
Total males	1,647	2,123	19
Percentage of males	26.3	50.4	.37
Percentage of separate experiments producing males	85.0	86.6	4.1

* P.F. = Paper filtrate. B.F. = Berkefeld filtrate. N.M. $\frac{1}{300}$ = One to three hundred dilution of normal medium.

ers per bottle regardless of whether or not all 10 mothers survived, since if deaths occurred in any bottle there was no way of telling when the mother or mothers died. The results of the four separate experiments of each series are totaled and tabulated under the series number in this table. Series 1, 2, and 3, being in the nature of preliminary experiments, are not as adequately controlled as to broods as are the rest of the experiments. It is questionable whether brood controls to insure experiments against male production on the part of normal mothers are necessary, since normal *Moina* mothers in adequate medium do not often produce males on first release; and in a series of experiments such as these the work is greatly increased by such controls to no great advantage, as the production of 0.37 per cent males by control mothers can have little, if any, significance in the interpretation of the experimental part of the work.

The 564 mothers in the irrigated bottles, bathed in from 200 to 850 ml. of flowing medium per mother, produced a total of 6,685 young, of which 26.3 per cent were males; whereas 555 mothers reared in unirrigated medium released 4,242 young, of which 50.4 per cent were males. Here, certainly, it is apparent that preventing the accumulation of excretory products by rearing mothers in flowing medium that is constantly being agitated, while it may have lessened, has not prevented the production of males by these mothers. A lower percentage of males was anticipated in the irrigated bottles than in the unirrigated experiments since, with irrigation, one has to contend with two or three factors irrespective of any excretory substances which may tend to reduce male production: namely, the constant renewal of the food and the mechanical agitation of both the medium and the mothers and, in addition, possibly aëration of the medium. That the food factor plays an important rôle in the experiment as a whole can easily be seen by comparing the average number of young released by mothers in both irrigated and unirrigated bottles, 11.8 and 7.6, respectively, with 21.5 the average young produced by the control mothers in normal medium. The same relation holds true for second eggs, though the mothers were transferred to normal medium when the first eggs were laid and presumably the number of second eggs would be determined by the normal environment. This serves to emphasize, as has already been pointed out by

Stuart, Tallman, and Cooper, that a mother may experience in an adverse environment some physiological injury which may carry over to the second release even though the animals are placed in normal medium before the second eggs are laid. It should be noted that these investigators made their observations on mothers reared in a medium too concentrated for the normal development of *Moina* mothers, whereas the present observations were made on mothers reared in a medium too dilute to permit normal development of the mothers.

DISCUSSION

In the three types of experiments reported in this paper an attempt has been made to rear *Moina* mothers under such conditions as would exclude the possible intervention of excretory substances in the control of sex. In the first experiment single mothers reared in as much as 500 ml. of highly diluted medium produced males, and it is very difficult to conceive of a single mother producing a sufficient concentration of excretory substances to influence in any manner her physiological activities, especially since the volume of normal medium must be reduced to approximately 7.5 ml. in order that an isolated mother may be influenced to produce males. On the other hand, it is quite improbable that decreasing the volume of medium to 7.5 ml. has reduced the total number of bacteria to the point where a mother experiences an actual shortage of food, provided the Cladocera can utilize as food all the species of bacteria present in the medium. Such evidence as there is on this point, however, tends quite strongly to show that the total count of viable bacteria is by no means a true criterion of the food value of a medium in question. In view of this it will not seem strange that no attempt has been made in these experiments to ascertain the bacterial content of the various dilutions of medium by direct count. We have even hesitated to associate differential counts (species enumeration) with male production in highly diluted medium or normal medium heavily crowded with *Moina* mothers, as evidence is not yet sufficient to warrant any definite conclusions from such a procedure. For example, we have been able to rear mothers in bacterized filtrates of normal medium containing several million bacteria per unit volume and have

observed that these animals apparently starved to death, while sister-mothers, reared in a similar medium with a similar number of bacteria but with an entirely different species, thrived normally. Until more is known regarding the species of bacteria that serve as food for Cladocera, total or differential counts of a medium, except under certain conditions, will only serve to confuse the issue. An extremely low count in a medium is doubtless a fair indication of the poor quality of the medium; but the reverse need not at all be true, namely, that a high count is indicative of a good medium. It is true that with a filtrate of aged normal medium bacterized with a known species a total count may be of considerable value over a limited period of time, but even here it must be remembered that at any time in such a medium a species of bacteria other than the one used for bacterizing may, in the course of several hours, completely overgrow the inoculated species. This is particularly true when Berkefeld filtrates are used as a base for bacterization, fine paper filtrates being more suitable for this purpose.

The tendency to view such media as hay or manure infusions as simple media containing a stable amount of food which, when diluted, will contain a proportionately diminished amount of food, has without doubt led to considerable error in the past. Thus Robertson (1923), working with hay infusions which were diluted a certain number of times with Ringer's solution, assumed and interpreted his results on the assumption that the actual food value (bacterial content) of the medium was reduced in proportion to the dilution over a period of 48 hours or more. Anyone familiar with the difficulties that attend the use of sterile balanced solutions, especially if they contain dextrose, will immediately recognize the fallacy of this reasoning. Furthermore, while the total viable count in these infusions may remain fairly constant over a period of time, the actual bacterial flora during this time may be constantly changing as some species pass through the logarithmic phase of growth to the period of decline while others are entering the logarithmic phase. Then, too, with the possible exception of the colon organisms, no method can yet be devised to make even a reasonably true differential count of all the species of bacteria present in such infusions. Add to this the fact that it has already been shown that not all species of bacteria

serve equally well as food for Cladocera, and one will easily recognize the tremendous complexity of the whole subject.

In a second series of experiments crowded mothers were reared in varying dilutions of concentrated manure infusions and transferred to fresh medium at 2-hour intervals over a period of from 18 to 24 hours previous to egg-laying. In the 1:12.5 dilution which is comparable to normal medium, but few males were produced; while in the higher dilutions (1:50 and 1:150), the percentage of males produced increased as the dilution increased. It is significant to compare the results of this experiment with those obtained by Stuart, Tallman, and Cooper when crowded mothers were reared in various dilutions of concentrated manure infusions and the mothers kept in the experimental bottles from early first instar to the time of release. Working with the same strain of *Moina* (Banta, line 1012) and the same type of medium as were employed in the experiments reported in this paper, these investigators found that crowded mothers reared in a 1:12.5 dilution of medium produced an average of 11.48 young per mother, of which 33.06 per cent were males. In our experiments crowded mothers reared in the same dilution of medium, but frequently changed to fresh medium before egg-laying, released 16.81 young per mother, of which but 2.41 per cent were males. It would not be at all difficult to ascribe the increase in average young per mother and the extremely low percentage of males in this experiment to the prevention of the accumulation of excretory substances due to frequent changing of the animals to fresh medium were it not for the fact that the same technical procedure repeated with a 1:50 dilution of medium gave results quite inexplicable on this basis. Mothers reared in a 1:50 dilution without being transferred to fresh medium produced an average of 6.13 young per mother, of which 74.87 per cent were males; whereas in the same dilution mothers transferred to fresh medium at the stated intervals released 14.78 young per mother, of which 35.05 per cent were males. Even more significant are the facts brought out by a comparison of the two experiments when a 1:150 dilution of the infusion was used. In this dilution crowded mothers reared without being transferred to fresh medium produced an average of only 4.73 young per mother, of which 17.32 per cent were males. This decrease in male percentage

in excessively dilute medium has been commented upon elsewhere in this paper. But when crowded mothers reared in a 1:150 dilution of medium are frequently changed to fresh medium, the percentage of males produced by such mothers is actually increased more than 300 per cent (53.82 per cent males having been produced). It would seem, then, that by rearing mothers in an environment in which there can be no significant accumulation of excretory substances, one may actually obtain an increase in the percentage of males over that obtained from rearing mothers in an environment which wholly favors the accumulation of excretory products. That this is not an accidental happening confined to the specified environment is shown in Table III, Experiments 9, 10, and 15, in this paper and will be shown again in a subsequent paper. It is admitted that the total number of mothers and the degree of crowding used in the two experiments compared are not equal; but it is felt that the comparison is nevertheless justified, especially since a greater degree of crowding was used by Stuart, Tallman, and Cooper than in the experiments herein reported.

It was also noted in this type of experiment that mothers reared in the 1:12.5 dilution of medium on three occasions laid eggs from 2 to 4 hours later than sister-mothers in the 1:50 dilution; but in every case the percentage of male young produced by these retarded mothers was less than that produced by the more rapidly developing mothers in the more dilute medium. The rôle of retardation in the production of males needs more investigation. That retardation and male production may be concurrent up to a certain point can be made a definite assertion; but that the retardation in itself is responsible for, or even casually related to, the appearance of males is certainly not true under some experimental conditions. Stuart and Banta (1931), and Stuart, Tallman, and Cooper (1931), have pointed out that rearing mothers in excessively diluted medium is conducive to female production even though such mothers are retarded in their development. On the other hand, moderate retardation caused by strong medium seemed to produce only females, whereas the same degree of retardation under conditions of moderate crowding would result in many males. In all the experiments reported by Stuart and co-workers not a single male offspring has been produced by a total

of 660 mothers reared in an excessively strong medium which caused a marked retardation in the mothers' development.

In a third series of experiments crowded mothers were reared in what has been termed "flowing medium." In these experiments it has been computed that mothers have been reared in the equivalent of from 200 to 850 ml. per mother of constantly changing medium and have produced males. It is difficult to conceive of an experiment wherein more assurance could be had that accumulation of excretory substances does not occur. In the last experiment (Series 15, Table III) in which 8.5 liters of medium were used in irrigating a single bottle, the total content of the bottle according to the rate of flow was changed slightly more than 120 times in the course of the experiment (approximately 28 hours); and this, together with the constant agitation of the medium previously described, must prohibit the accumulation of any substances as transitory and unstable as the metabolic products of Cladocera have been assumed to be (Banta and Brown, 1929*b*). The experiment under discussion is another example of the production of a high percentage of males under conditions eliminating the accumulation of excretory substances while sister-mothers reared in an environment favoring their accumulation produces a much lower percentage of males.

It is felt that, despite the success attending the production of males by isolated mothers in large volumes of medium (Table II), the production of males by crowded mothers in flowing medium is easier and more certain of success even when 600 ml. or more medium is used per mother. The significance of this, if it is indeed true, is not clear. We cannot feel, however, that the crowding results in the accumulation of excretory substances which might be conducive to the control of sex in these experiments. On the other hand, any theories regarding the effect of contiguity of the animals alone would be mere conjecture at this time, though it is a possibility not to be completely overlooked or ignored.

In considering the work as a whole, it seems conclusive that males have been produced by mothers reared in dilute medium under conditions which would prohibit the accumulation of transitory and unstable metabolic products of the Cladocera. How, then, are these findings to be explained at the present time on any basis other than

an inadequacy of available food? It is feasible that in diluting the normal medium some factor other than direct diminution of food may function in the reaction. For example, dilution of the medium may bring about an insufficiency of a possible accessory food factor (Stuart and Banta, 1929) which in some way may be associated with the control of sex in these animals.[1] But until such a factor is demonstrated, the term "diminution of food" will be used in a broad sense. A repetition of some of the experiments pertaining to food as a sex-controlling agent with bacteriologically sterile mothers in pure cultures of their appropriate food would doubtless add considerably to our knowledge of the subject.

Certain aspects of our experiments tend to the conclusion that crowding, in some way other than reducing the food, may be a sex-controlling agent; but it is not at all clear how crowding other than favoring the accumulation of excretory substances can exercise a sex-controlling action. Crowding under some conditions may be associated with some other aspect of the physiological effects of "aggregation" (Allee, 1931), since we have casually noted that crowding 10 mothers in 60 ml. of normal medium seems to stimulate aggregation on the part of the mothers more than the same degree of crowding in dilute medium. We are not yet prepared to dismiss the factor of crowding as a sex-controlling agent under all conditions; but we do question whether excretory substances of the transitory and un-

[1] Since the completion of this manuscript, our attention has been called to some work pertinent to this aspect of the question by van Herwerden (1923), who found that extracts of adrenal capsule added to a medium in as small as ±1 mg. amounts had a marked effect on the growth and reproduction of *Daphnia pulex de Geer*. Growth of the animal is greatly stimulated, number of young per brood and number of broods per mother are increased; sexual reproduction and males are suppressed. Evidence is offered to show that the extracts do not act primarily to increase the food in the medium as far as Algae are concerned, but unfortunately the bacteriological aspect was not considered. The work as a whole, however, strongly indicates that food accessory factors may play some rôle in the sex life of these animals.

The statement made by Stuart and Banta (1929) regarding the possible rôle of an accessory food factor in the growth of Cladocera was formulated quite independent of the literature. It would perhaps be well to mention here that the senior author and writer of this series of papers entered this field of investigation (sex control) as a bacteriologist and that these previous reports were not overlooked or ignored intentionally but were omitted due to a lack of knowledge of the literature on the subject as a whole at that time.

stable nature described by Grosvenor and Smith (1913), Smith (1915), and Banta and Brown (1929*b*) can under any circumstances determine the sex of the offspring produced by isolated or crowded cladoceran mothers, which, after all, brings us back to the conclusion regarding the importance of food in this connection originally reached by Issakowitsch (1905), Woltereck (1911), and others.

CONCLUSIONS

1. Isolated *Moina* mothers reared in as much as 500 ml. of highly diluted medium will occasionally produce males. Small broods of young and retardation of mothers, as well as dilution of medium, make it seem probable that the production of males is due to lack of food.

2. Crowded mothers reared in various dilutions of medium and frequently changed to fresh medium to prevent the accumulation of excretory substances produce males in accordance with the dilution of the medium.

3. Crowded mothers reared in constantly flowing medium will produce males provided that the medium used in the experiment be sufficiently dilute.

4. Under the conditions specified in this paper the sex of the young produced by the crowded mothers must be due to a lack of food together with any other effects that might be caused by diluting the medium in which the animals are reared, or to any effects caused by crowding the animals other than the accumulation of the *transitory* and *unstable* excretory substances described by various writers.

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STUDIES ON THE SEXUAL CYCLE IN BIRDS. VI. EFFECTS OF WHITE, GREEN, AND RED LIGHTS OF EQUAL LUMINOUS INTENSITY ON THE TESTIS ACTIVITY OF THE EUROPEAN STARLING (STURNUS VULGARIS)[1]

(Four plates)

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PREVIOUS studies on the sexual cycle of the male starling (*Sturnus vulgaris*) dealt with the normal sexual cycle in nature and with results of experimental modification of daily light and exercise periods using 60-watt electric-light bulbs at controlled distance and periods, with both experimental and natural controls (Bissonnette, 1930*a*, 1930*b*, 1931*a*; Bissonnette and Chapnick, 1930). A recent report of the results of experiments with intensities of illumination by white electric light after sunset, using bulbs of from 10 to 60 watts at the same distance, with controls, shows the effects to depend on the strength of the bulbs used, if the period of illumination is constant (Bissonnette, 1931*b*), and the intensity of illumination is below that of a 25-watt bulb at a distance of 9 inches (about 16.5 foot-candles).

Results indicate that the relative daily light period conditions the breeding activity of the starlings as well as that of the junco reported on by Rowan (1925, 1926, 1928, 1929), so far as increase and decrease of period causing progressive or regressive testis changes respectively, is concerned. However, in nature, regressive changes set in before June 21, while the days are still growing longer. The reason for this has not been yet determined, though data bearing on it are being secured.

It was shown that relative, and not absolute, daily light period conditions these changes (Bissonnette, 1931*a*). Relative states of the testes of two sets of birds, kept for a month in the same cage on identical light rations and other environmental conditions, become

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reversed if one set has previously been brought to high activity by larger light rations per day and the other has remained less active under smaller daily light ration.

Increased daily periods of muscular exercise cause a lag in the appearance of light-induced changes, followed by quicker reaction, so that the initial delay is more than caught up later and birds with increased exercise periods finally surpass those without them in the degree of change in spermatogenic activity, either progressive or regressive. Longer exercise periods lead at first to increase in intertubular pigmentation and may or may not maintain the excess. Increased exercise periods do not induce increased spermatogenic activity in the absence of increased light periods in the starling as it is reported to do with the junco (Rowan, 1928).

Testes may be maintained, for periods of at least a month, in a constant condition of partial regression by maintaining constant light and exercise periods (Bissonnette, 1931*a*) and constant light intensity (Bissonnette, 1931*b*).

There is a difference in rate and degree of response to light changes between the testes of young birds of the previous summer's broods and those of birds which have been sexually mature at least once before. Mature birds, as a rule, respond more quickly and fully to progression-inducing light changes; young ones, to regression-inducing ones, if they have already increased in activity for the first time.

This paper is a report of investigations into the effects of light of different wave-lengths or spectral colors, from different regions of the visible spectrum, as compared with unfiltered light from an incandescent 15-watt bulb. The intensity of illumination of each light was reduced to a standard intensity by filters and corrections for distance based on the law of inverse squares. The study is an attempt to evaluate the effectiveness of light from different parts of the visible spectrum from these incandescent bulbs, on a basis of equal intensity of illumination.

MATERIAL AND METHODS

The birds used were European starlings (*Sturnus vulgaris*) caught in the same place and maintained, in general, in the same manner as described in a previous paper (Bissonnette, 1931*b*). It was hoped

that red, green, and violet filters could be used with such light intensities as would insure the transmission of equal intensities of illumination to the birds. Since it was known that 10-watt bulbs at a distance of 9 inches would induce slow increase in testis activity of these birds on increasing daily light rations (Bissonnette, 1931*b*), it was hoped to get the luminous equivalent of a 15-watt bulb at a suitable distance for all three colors. For violet this has so far been impossible, because the available filter of known percentage of light transmission—the "Violet Special" of the Corning Glass Company (makers of all the filters used)—transmits only 0.113 per cent of the light intensity; even 1,500-watt bulbs could not give the required intensity beyond the filter, and the heat generated cracked the filter and threatened to set fire to the apparatus. However, some birds have been subjected to violet light, using this filter and a 1,000-watt bulb at a safe distance of 29 inches, delivering only about 12.4 per cent as intense light at the roost as that used with the other lights, or about 0.32054 foot-candles of violet.

This violet-light experiment was not begun till the ones on red, green, and white light were well under way, on March 5. It furnishes a test as to whether violet light of low intensity of illumination is capable of inducing testis activity in these birds. So its results are included in this paper, though they are not as accurately controlled and do not give reactions comparable with those for the other lights. It is hoped that at some future time arc lights may be tried with the violet filters at intensities equal to those used with the other colors, when a suitable apparatus has been devised.

In all previous experiments in this laboratory the illumination has been from the side. For this work it was found necessary to light from above, to permit ventilation and prevent light from one source from interfering with that from others and vitiating the results. It was also easier to control the distance from the source of light to the roost center, to deliver the intensity desired to the birds. Since the birds were free to move about in the cages, the light intensities upon them at different times varied. The habits and roosting places of the birds with reference to the center of the roost, where the standard intensity of illumination was delivered, undoubtedly cause variations in the total intensity of illumination received by different birds in the same cage; but all cages were treated alike in this respect,

and no significant differences in behavior of the birds in any cages were noted. All seemed to circulate from one position to another during light periods; so the birds as a whole in any one cage are comparable to those in any other, though two birds in the same cage may have effective differences in total light received during any period.

There were 112 birds used to start these experiments, divided, indiscriminately as to sex, among seven cages, on January 28, 1930, immediately after completion of tests on the effects of different intensities of unfiltered electric light. Some few, appropriately ringed, birds were carried forward into this series from the previous one. Reports on their reactions are included in this paper.

Two cages were equipped with $6\frac{1}{2} \times 6\frac{1}{2}$-inch square filters, Nonex H. R. Pyrometer Red, unpolished, transmitting 3.5 per cent of light intensity, set in light-tight frames in the roofs of the cages. A 200-watt electric-light bulb was set above the filter, with its filament at 22.5 inches from the center of the roost and screened from other cages by a large-sized pipe of galvanized iron open at the top and having small chinks at the bottom to admit air currents for cooling. Above the filter, with air space between, was placed an equal-sized square of ordinary window glass, of the same sort as was used in the cages that received otherwise unfiltered light, where it prevented escape of birds.

In these two "red-light" cages, the light intensity at the roost center closely approximated 114.8 lumens acting at 22.5 inches or 2.5975 foot-candles, if the window glass effect is neglected.

Two "green-light" cages were similarly equipped with Sextant Green, unpolished filters, transmitting 8.75 per cent of the luminous intensity of a 100-watt bulb set at 22.7 inches, about 117.25 lumens at the mean effective distance, or 2.6064 foot-candles, approximating closely the intensity of illumination at the roost center in the other light cages.

A fifth cage was similarly equipped except for the absence of any filter below the window glass in the roof, and gave 131 lumens at a distance of 24 inches, or 2.6051 foot-candles, if the window glass is neglected as before. This closely approximates the intensity of illumination at the roost center given in the other light cages.

Two similar cages of birds were used as controls without added

direct light but with the semidarkness or faint light reflected from the ceiling over the other cages. So far as this reflected light is concerned, all cages were alike.

There were sixteen birds in each of the lighted cages, and the rest of 112 birds were divided between the two control cages. In capturing these birds, ten were smothered in the box in bringing them into the laboratory and used as outside controls for the end of the preceding experiment and the beginning of this one. Of these, three were females and seven males. A few more birds of the lot died within a week, after which no more deaths occurred; those that died are not included in this study.

As in previous experiments, light was freely admitted to the room by three large windows during the day, and blinds were closed only in later stages of the experiments as described below.

All lights were turned on after nightfall for 1 hour on January 28, and this time was increased ½ hour per night till 6 hours of artificial lighting were reached. The light intensity was then kept constant till February 14, when it was increased to 6½ hours and to 7 hours on February 16, then maintained at 7 hours per night. An electric time switch was used, which controlled all the lights. This 7-hour period of added light was superimposed upon the normally slowly increasing day length from the beginning of the experiment till March 5, after which the room was curtained closely from about 4:45 P.M. until 7:00 A.M. nightly. At this latter date violet-light treatments were begun in one of the control cages, with similar setup and Violet Special filter as mentioned above, and on the time schedule already reached.

Food trays were removed from all cages from about 4:45 P.M. until 7:00 A.M., to equalize feeding periods for all birds.

When birds were killed for gonad study, testes were carefully measured to the nearest tenth of a millimeter in length and thickness with fine calipers, immediately on removal from the birds. Ovaries were not measured, but their relative condition as to size of follicles and the condition of the oviducts were tabulated. Both sorts of gonads were fixed and treated with the same technique as before (Bissonnette, 1931*b*) and sectioned 7.5 μ thick. Left testes were used for study.

On March 17, one red-, one green-, and the white-light cage were cleared of birds by moving the "reds" and "greens" into one cage each, with the whites remaining in the "control" cage, properly marked. These birds were continued with treatments as before.

On March 18, fifty-four birds from outside were divided up indiscriminately into the freed "red," "green," and "white" cages and started on 7-hour treatments similar to those of the original birds, to test the effects of immediate change to the maximum treatment and persistence in it, added to a $9\frac{3}{4}$-hour daily daylight period. These birds had their daily exposure to normal daylight reduced $2\frac{1}{2}$ hours per day.

The follicles of the ovary of one female bird from outside, killed on this date, had started to enlarge.

"Inside controls" were not run on these newly added birds. Those from the experiments of the previous year may be used for comparison, since their time schedule was practically the same as for these, and it was carefully checked (Bissonnette, 1931*a*).

Both original and newly added birds were killed as available on the subsequent dates of killing and are described in Tables IV, V, VI, VII, and VIII. Birds killed on March 17 and before are described in Tables I, II, and III. The "violet-light" birds on about 0.32054 foot-candles, in one of the original "control" cages from March 5 onward, with schedule like those of other light-treated birds for the same period, are included in Tables III and IV. Birds carried over from previous experiments, with the data concerning them, are included with other birds killed on the same dates.

RESULTS—GENERAL FINDINGS AND APPARENT EXCEPTIONS

1. On February 15, after 19 days, testes of birds subjected to the red- and white-light treatments, as described above (Table I), had been stimulated to increasing spermatogenic activity (those with white more than those with red); those subjected to green were slightly inhibited as compared with controls. However, birds, brought to complete spermatogenic activity by previous light treatments of about seven times as great luminous intensity, when treated with white light in this experiment, underwent regression on the reduced intensity and period of treatment. This is in accord with

results of previous experiments on the relation of duration and intensity of daily light exposures to testis activity in these birds.

2. On March 1, after 33 days, red and white light continue to stimulate testes to progressive changes (red more than white); green

TABLE I

BIRDS KILLED FEBRUARY 15, 1930

Cage	Age*	Gonad Size† (Mm.)	Color	Days in Cage	Remarks
Controls	Y.	R. 1.8×1.3 L. 2.3×1.4	Dark green	19	Fig. 1.—Slight signs of activity of germ-cells; tubules slightly enlarged; no synizesis stages.
Controls	M.	R. 3.0×2.3 L. 3.7×2.0	Dark green	19	Fig. 2—Tubules larger than foregoing; mitosis and spermatogonia numerous; interstitials flattened.
15-watt white light	Y.	R. 5.4×3.6 L. 0.5×3.7	Lighter	98	Fig. 5.—Had 15-watt light in previous experiment; spermatogenesis complete; spermatids and spermatocytes few; some regression from Jan. 27, likely due to initial reduction of light.
15-watt white light	Y.	R. 3.3×2.5 L. 4.0×2.2	Dark	19	Fig. 3.—Tubules and germ-cells slightly advanced over Fig. 2; few synizesis stages.
15-watt white light	M.	R. cut×2.8 L. 4.4×2.6	Dark	19	Fig. 4.—Advanced a little beyond Fig. 3.
Red light	Y	R. 2.5×1.9 L. 2.7×1.9	Dark	19	Fig. 1.—Less advanced than testes of "white-light" birds, but more than controls (very slightly); few synizesis stages.
Red light	M.	R. 3.5×2.4 L. 3.3×2.2	Dark	19	Fig. 1.—Too slightly advanced over foregoing to show.
Green light	Y.	R. 2.8×2.1 L. 3.4×2.0	Dark	19	Fig. 1.—Tubules slightly less advanced than "red-light" birds; very similar.
Green light	M.	R. 3.2×2.3 L. 3.8×2.1	Dark	19	Fig. 6.—Show decided regression; vacuoles in the tubules; some mitoses; interstitials not flat.
Red light	M.	R. 8.8×6.3 L. 9.9×5.9	Light	8 days after 79 days at 25-watt white at 9 inches distance; died February 4/30	Fig. 17.—Undergoing regression; clear Sertoli cell outlines, necrotic nuclei, interstitials not rounded; little change in week of red light from the condition of Jan. 27 (Bissonnette, 1931*b*).

* M. = mature bird; Y. = juvenile bird.

† R. = right testis; L. = left testis.

induces regressive changes. These red-stimulated testes reach complete spermatogenesis in one young bird; but in a mature bird from the same cage the amount of stimulation is less than that in white-lighted birds. This apparent exception may be due to the possibility that the particular bird studied roosted habitually near the ends of the roosts, farther from the light, and in less intense illumination. No such habits could be noted, though the birds were carefully watched (Table II).

A bird from the previous experiment, like the one described above killed on February 15, was still undergoing regression on March 1, and had reached the early July condition of the normal cycle.

3. On March 17, after 49 days under the conditions of this experiment, red light had induced complete spermatogenesis in all birds'

TABLE II

BIRDS KILLED MARCH 1, 1930

Cage	Age*	Gonad Size† (Mm.)	Color	Days in Cage	Remarks
Inside control	M.	R. 5.0×3.4 L. 5.3×2.7	Dark	33	Fig. 8.—Just showing first synizesis stages; compare with Fig. 4.
Inside control	Y.	R. 3.0×2.4 L. 3.0×2.2	Dark	33	Fig. 7.—Stages like the foregoing, but tubules smaller.
Green light	M.	R. 3.6×2.4 L. 4.6×2.2	Dark	33	Fig. 2.—Not so far advanced as controls; rare synizesis stages, retarded by green light(?)
Green light	Y.	R. 2.7×1.9 L. 3.3×1.6	Dark	33	Fig.13.—Similar to Fig. 2, but slightly smaller tubules; interstitials still recognizable.
Red light	M.	R. 5.1×3.6 L. 6.6×3.2	Dark	33	Fig. 12.—Not quite so far advanced as Fig. 4, above; probably roosted in dark corner of cage; not quite so far advanced as "white-light" birds.
Red light	Y.	R. 9.0×7.6 L. 10.4×6.6	Light	33	Fig. 11.—Complete spermatogenesis and two-thirds maximum size; April condition on normal cycle.
White light	M.	R. 4.6×3.2 L. 5.6×3.2	Dark	33	Fig. 4.—But slightly in advance of condition at last killing.
White light	Y.	R. 3.2×2.5 L. 3.8×2.0	Dark	33	Fig. 10.—Tubules slightly enlarged since last killing; no advance in germ-cell stages; not so far advanced as Fig. 4.
White light	M.	R. 3.0×2.5 L. 4.0×2.2	Dark	112	Fig. 9.—Had been in 15-watt cage at 9 inches distance in the preceding experiment; has undergone definite regression from condition on last killing date (Fig. 5); only spermatogonia remain of the germ-cells.

* M = mature bird; Y. = juvenile bird.
† R. = right testis; L. = left testis.

testes, and some (young ones') had passed the climax and regression had begun; but equal intensities of neither white light, which is stimulating, nor of green, which is inhibitory in effect, brought about complete spermatogenesis (Table III).

Whether the regression of the young "red-light" birds is wholly due to the reduction of daylight period on March 5, to which the young birds are more susceptible than the mature, or to inability to remain at the climax of spermatogenesis, or to both, is not yet clear.

Violet light, of the intensity used, in the 12 days before March 17, neither stimulated nor inhibited testis activity appreciably.

4. In birds of the original lot killed on March 29 after 60 days (Table IV), it was found that neither red, green, nor violet light prevented the testes from undergoing regression, though the regression of the "red-light" birds was from a climax of activity which may possibly be difficult to maintain. "Red-light" birds regressed to greater degrees than "green-light" ones. This regression in all birds

TABLE III

BIRDS KILLED MARCH 17, 1930

Cage	Age*	Gonad Size† (Mm.)	Color	Days in Cage	Remarks
Inside control	M.	R. 3.0×2.4 L. 3.7×2.3	Light green	49	Fig. 1.—But slightly in advance of March 1 condition; little if any effect of reduced light ration is evident.
Inside control	M.	R. 4.0×2.8 L. 4.5×2.7	Light green	49	Fig. 4.—As above described for Fig. 1.
Violet	M.	R. 3.8×2.8 L. 4.1×2.4	Light green	38 control+11 days violet	Fig. 2.—Not so far advanced as controls above, in most respects, but within limits of controls.
Violet	Y.	R. 3.8×2.8 L. 4.3×2.5	Light green	38 control+11 days violet	Fig. 2.—As above; neither is appreciably stimulated nor retarded as compared with controls.
Green light	Y.	R. 3.7×2.6 L. 4.4×2.2	Light green	49	Fig. 15.—Very far regressed; tubules very small; vacuoles in some; necrotic nuclei; rounded interstitial cells; October condition of normal cycle.
Green light	M.	R. 4.4×2.9 L. 5.0×3.1	Light green	49	Fig. 16.—Not so far regressed as the foregoing, but very definitely so; vacuoles in cytoplasm.
Red light	M.	R. 9.6×6.9 L. 11.3×7.1	Gray	49	Fig. 5.—Complete spermatogenesis; two-thirds maximum size; young sperms; all stages of germ-cells; advanced since March 1.
Red light	Y.	R. 5.5×3.3 L. 5.7×3.3	Light green	49	Fig. 16.—Regressing; on March 1 these birds had been at complete spermatogenesis like Fig. 5.
White light	M.	R. 4.4×3.7 L. 4.8×3.0	Light green	49	Fig. 16 } Regression is evident in both, due to light reduction of 1¾ hours, March 1.
White light	M.	R. 3.6×2.6 L. 4.2×2.3	Dark	49	Fig. 8 }

* M. = mature bird; Y. = juvenile bird.
† R. = right testis; L. = left testis.

is doubtless induced by the 1¾ hours' decrease of normal daylight per day on March 5. However, there are some indications that young "red-light" birds may resume activity to some extent after their initial regression.

The greater regression of "red-light" birds from complete spermatogenesis suggests that later stages of germ-cells (sperms, spermatids, and both secondary and primary spermatocytes) are more susceptible to regression-inducing light-ration changes than are the earlier

stages; and that red light enables the earlier germ-cell stages to resist the effects of reduced total light.

5. In birds of the second lot, introduced on March 18 and killed on March 29, after 11 days, having their normal daylight exposure reduced by 2½ hours, and with 7 hours of added artificial light per day for that period, "white light" may or may not permit regression; "red-light" birds regress more than "green-light" ones, and begin progression after clearing up débris in their tubules more quickly than "green-light" ones (Table IV).

Only in the case of "white-light" birds was complete spermatogenesis reached in this lot of birds at this time. Considerable irregularity in effects is evident.

The degree of testis advancement toward complete activity for mature birds in general under these last conditions is for white, green, red, in descending order. For young birds under similar treatments, it is green, red, white, with but little difference between the last two. All except mature "white-light" birds show slight regression or delay in spermatogenic activity.

6. On April 12, after 74 days, testes of birds from the original lot show that red light restores activity completely after reduction of day length; green does not. But testes of "green-light" birds after rapid regression may reach a state of equilibrium slightly in advance of controls. "Controls" are slower in clearing out débris from tubules than "green-light" birds. Red is stimulating, green inhibitory, and controls intermediate (Table V).

7. On April 12, after 25 days, testes from birds of the second lot show that neither red, green, nor white light of the intensity used can prevent regression under the reduced daylight ration given these birds. However, white light appears to have delayed and slowed the regression much more than red or green. Red light has permitted the most regression of the three. "Green-light" birds have reached the condition found on March 10–15 of the normal cycle (Table V).

Seven hours of treatment with the intensity of light used here, (about 2.6 foot-candles) does not compensate for loss of 2½ hours of daily daylight. It more than did so when 60-watt bulbs were used at a distance of about 9 inches yielding about 30.34 foot-candles (Bissonnette, 1931*a*). This difference in results, when different in-

tensities of illumination are used, is in accord with a study of the effects of different intensities (Bissonnette, 1931*b*).

The difference in degree of regression, with different colors of light, shows that, with the same age of bird, the effect on testis activity

TABLE IV

BIRDS KILLED MARCH 29, 1930

Cage	Age*	Gonad Size† (Mm.)	Color	Days in Cage	Remarks
Inside control	M.	R. 3.7×2.8 L. 4.5×2.7	Green	60	Fig. 18.—Some little advance from mature birds on March 17; tubules enlarged; more synizesis stages; rare primary spermatocytes.
Red light	M.	R. 4.5×3.3 L. 5.0×2.7	Green	60	Fig. 12.—Regressed from March 17 condition (Fig. 5); tubules lack lumina; no germ-cells beyond stage of synizesis; interstitials rounding up.
Red light	Y.	R. 5.3×3.3 L. cut across	Green	60	Fig. 18.—Not so far regressed as the foregoing; still have few spermatocytes; synizesis stages many; have advanced since March 17.
Green light	M.	R. 4.2×3.3 L. 4.4×2.5	Green	60	Fig. 4.—Smaller than controls and have more primary spermatocytes; not so many synizesis stages; smaller than violet-treated-birds testes; regressed.
Green light	Y.	R. 2.9×2.0 L. 3.4×1.7	Green	60	Fig. 6.—Smaller than the foregoing; more regressed; vacuoles in tubules; germ-cells reduced in number.
Violet	M.	R. 3.2×2.1 L. 3.3×2.0	Green	24	Fig. 2.—Smaller than controls, tubules have fewer germ-cells; little change since March 17; fewer mitoses than then or than in controls.
Violet	Y.	R. 3.5×2.5 L. 4.1×2.3	White	24	Figs. 19, 20.—Like controls but testes are both almost without pigment; more synizesis stages than in mature bird above; abnormal tubule arrangement.
White light	M.	R. 12.8×8.5 L. 12.9×8.5	White	11	Fig. 24.—Spermatogenesis complete to young sperms; no regression evident; little farther advanced than birds on normal cycle.
White light	Y.	R. 4.4×3.0 L. 4.8×2.6	Green	11	Fig. 25.—Not so far advanced; no germ-cell stages beyond primary spermatocytes; regression evident.
Green light	M.	R. 6.3×5.0 L. 7.7×5.7	Light	11	Fig. 17.—Decided regression, shown by Sertoli cell outlines, but increased size and advanced over March 18 condition.
Green light	Y.	R. 6.0×4.7 L. 6.4×4.7	Light	11	Fig. 17.—As above. Both have spermatocytes and synizesis stages of germ-cells.
Red light	M.	R. 6.2×4.8 L. 7.3×4.4	Light	11	Fig. 22.—Not so far advanced as Fig. 17 above; the signs of regression are not so pronounced, but present, nevertheless.
Red light	Y.	R. 4.3×3.1 L. 5.2×2.8	Green	11	Fig. 23.—Less advanced than Fig. 22; regression is evident; Sertoli cell outlines have disappeared.

* M. = mature bird; Y. = juvenile bird. † R. = right testis; L. = left testis.

depends on the wave-length of the light used in experimental treatments to make up for daylight loss.

8. On April 28, after 90 days, testes of birds from the original lot

show that, while "green-light" birds have remained without change during 16 days since the last time of killing, under constant light conditions, "red-light" birds have resumed progression after the

TABLE V

BIRDS KILLED APRIL 12, 1930

Cage	Age*	Gonad Size† (Mm.)	Color	Days in Cage	Remarks
Inside controls	Y.	R. 3.3×2.3 L. 3.8×2.3	Dark green	74	Fig. 6.—Not so far advanced as on March 29 (Fig. 18); synizesis stages rare and abnormal; necrosis is evident; vacuoles in some tubules, not in others,
Inside controls	Y.	R. 3.7×2.8 L. 4.4×3.0	Dark green	74	Fig. 8.—Same as the foregoing, but tubules larger.
Green light	M.	R. 4.3×2.5 L. 4.4×2.3	Dark green	74	Fig. 14.—Have regressed since March 29 and cleared up débris in tubules; mitoses present; synizesis also; becoming active again.
Green light	Y.	R. 3.3×2.5 L. 3.3×2.2	Dark green	74	Fig. 8.—Similar to the foregoing but with fewer germ-cell nuclei and synizesis stages in tubules; also becoming active or maintaining themselves.
Red light	M.	R. 8.9×5.9 L. 11.7×6.2	Light	74	Fig. 21.—Progressed since March 29; all stages of germ-cells up to secondary spermatocytes; lumen in each tubule; interstitials indistinguishable.
Red light	Y.	R. 10.8×7.8 L. 13.0×7.1	Light	74	Fig. 11.—All stages of germ-cells to mature sperms; characteristic breeding condition; have progressed since March 29.
White light	M.	R. 7.9×5.4 L. 9.7×5.5	Light	25	Fig. 17.—Regressed since March 29 but not very much; débris is present in some tubules, not others; the Sertoli cell outlines reach centers of tubules.
White light	Y.	R. 3.3×3.0 L. 4.3×2.7	Dark green	25	Fig. 16.—Farther regressed than the foregoing; all cell generations beyond synizesis have sloughed into the lumen of tubule here and there.
Green light	M.	R. 4.9×3.3 L. 5.0×2.7	Dark green	25	Fig. 14.—Regressed since March 29 but not so much as "white"-treated bird above; débris is cleared up; has gone back to condition in nature for March 15; second testis was lost and not sectioned.
Green light	M.	R. 4.3×2.9 L. 4.9×2.7	Dark green	25	
Red light	M.	R. 3.0×2.3 L. 4.0×1.6	Dark green	25	Fig. 8.—Tubules smaller than in bird above; the débris is cleared up and testis is resuming its germ-cell activity.
Red light	Y.	R. 3.2×2.0 L. 3.7×2.0	Dark green	25	Fig. 26.—Tubules smaller than in the foregoing, but germ-cell activity greater and synizesis stages more numerous; size reduced since March 29.

* M. = mature bird; Y. = juvenile bird.

† R. = right testis; L = left testis.

light-ration decrease and have reached complete spermatogenesis, in the case of young birds, and are nearing it in mature birds (Table VI). Young birds are more susceptible to the stimulating red light as here used than mature ones are. Birds under identical conditions

remain for 16 days, or longer, in about the same state of testis activity, once the previous light changes have become completely effective.

9. On April 28, after 41 days, testes of birds from the second lot (Table VI) show that both "red" and "white-light" birds have re-

TABLE VI

BIRDS KILLED APRIL 28, 1930

Cage	Age*	Gonad Size† (Mm.)	Color	Days in Cage	Remarks
Green light	M.	R. 3.8×2.8 L. 4.4×2.7	Dark green	90	Fig. 14.—About same size as on April 12, light was constant and so were testes.
Green light	Y.	R. 3.6×2.7 L. 4.2×2.0	Dark green	90	Fig. 23.—Same as the foregoing.
Red light	M.	R. 7.6×5.5 L. 8.2×4.7	Light	90	Fig. 29.—Stages only up to primary spermatocytes; Sertoli cell outlines evident; regression has occurred; but they have progressed since April 12.
Red light	Y.	R. 11.5×8.2 L. 12.3×8.0	Light	90	Fig. 11.—These testes have reached complete spermatogenesis after reduction of light; the regression has been prevented or very slight.
Red light	M.	R. cut×3.2 L. 5.5×3.0	Dark	90	Not studied histologically.
Green light	Y.	R. 3.6×2.6 L. 4.1×2.2	Dark	169	Fig. 28.—Carry-over from 60-watt cage of preceding experiment; regressed from complete spermatogenesis to condition of early autumn; very small tubules.
White light	M.	R. 4.7×3.1 L. 5.0×2.8	Dark	41	Fig. 23.—Stages only to few primary spermatocytes advancing to spermatogenesis, from state of Fig. 17.
White light	Y.	R. 11.4×8.6 L. 13.5×7.5	Light	41	Fig. 5.—Advanced to complete spermatogenesis after initial regression to condition of Fig. 16.
Green light	M.	R. 4.5×3.0 L. 5.5×3.3	Dark	41	Fig. 31.—Still regressing and clearing up débris.
Green light	Y.	R. 3.3×2.3 L. not found, none there.	Dark	41	Fig. 1.—Regressed to late autumn condition. The interstitials are vacuolated, as germ-cells or Sertoli cells were previously.
Red light	M.	R. 5.2×4.0 L. 5.5×3.5	Dark	41	Fig. 27.—More advanced than "white"-treated bird of same lot; has advanced since April 12; regression has been lessened by red light; primary spermatocytes present.

* M. = mature bird; Y. = juvenile bird.

† R. = right testis; L. = left testis.

sumed progression and "red-light" birds have caught up to "white-light" ones of the same age, and surpassed them. Young birds are farther advanced than mature ones. "Green-light" birds are still undergoing regression. Vacuoles like those previously appearing in the cytoplasm inside the tubules now appear in the interstitial cells. This is in accord with the conclusions of Orban (1929) that in the

crow such vacuoles are filled with lipoid globules, which pass out from the interior of the tubules to the interstitial cells, during regression of germ-cells, and react to acid-fixing agents in the same way in both situations.

Again, with these birds at this time, the descending order of stimulation effects upon testes is red, white, green, with the first

TABLE VII

BIRDS KILLED MAY 8, 1930

Cage	Age*	Gonad Size† (Mm.)	Color	Days in Cage	Remarks
Green light	M.	R. 3.8×2.7 L. 4.1×2.1	Dark	100	Fig. 30.—Regressed since April 28, on green-light ration; no germ-cells beyond spermatogonia present; mitoses present.
Green light	Y.	R. 3.9×2.8 L. 4.1×2.3	Dark	100	Fig. 2.—Not so far regressed as the foregoing; débris is cleared up in tubules; mitoses present; still about the same stage as on April 28 (Fig. 23).
Red light	M.	R. 13.0×9.2 L. 15.0×7.9	Light	100	Fig. 11.—Still at complete spermatogenesis; slightly in advance of stage on April 28 (Fig. 29).
Red light	Y.	R. 2.9×1.7 L. 3.2×1.6	Dark	100	Fig. 28.—Very minute tubules; has passed the crest of spermatogenesis and gone far toward complete regression.
Green light	M.	R. 3.3×2.4 L. 3.7×2.1	Dark	179	Fig. 32.—Had been subject to 60-watt bulb in preceding experiment; far gone in regression; few synizesis stages remain; have come to late autumn condition.
Green light	Y.	R. 3.7×2.6 L. 3.9×2.3	Dark	179	Fig. 12.—Not so far regressed as preceding; only a few synizesis stages remain; débris all cleared up.
White light	M.	R. 3.7×2.9 L. 4.1×2.3	Dark	51	Fig. 30.—Regressed since April 28; no synizesis stages; mitoses present; nuclei densely packed in tubules; interstitial cells rounded and recognizable.
Green light	M.	R. 4.6×3.1 L. 4.9×2.9	Dark	51	Fig. 16.—Regression very evident; vacuolated cytoplasm; necrotic nuclei; no germ-cells beyond very few primary spermatocytes; regression has been slow, since April 28.
Green light	Y.	R. 2.7×2.3 L. 3.0×1.8	Dark White	51	Figs. 33–36 inclusive.—Left testis almost without pigment; right normally pigmented; midwinter stages; regression practically complete.

* M. = mature bird; Y. = juvenile bird.

† R. = right testis; L. = left testis.

two stimulating and the last inhibitory, for birds of the same general age groups. Effects of the initial decrease of light ration are passing off.

10. On May 8, after 100 days, testes of birds from the original lot (Table VII) show that, while "green-light" birds are still neither progressing toward spermatogenesis nor regressing, mature "red-light" birds are still at the climax of spermatogenic activity and

young ones regressing from this climax, reached some time before April 28. Again the difference in reaction of young and mature birds appears. Regression in this case does not appear to be due to the action of red light, as such, but rather to an inability of the birds to hold to the climax of spermatogenesis for long, just as they fail to do in nature before June 15 (Bissonnette 1930*a*, 1930*b*). This phenomenon is being studied and will be reported upon later.

Again, with the possible exception of the young "red-light" bird just described, gone over the crest of activity, red light proves more effective in stimulating testis activity than green.

11. On this same date, after 51 days' treatment, testes of birds from the second lot (Table VII) show that "green-light" birds have continued regression while "white-light" ones have passed over the climax of spermatogenesis, which they reached about April 28, and are undergoing regression. This is probably due to a failure to be able to remain at the climax for very long, just as was suggested for "red-light" birds of the original lot on this same date. White light is more stimulating than green.

12. No "red-light" male birds were killed at this time from this lot, as there was but one left in the cage and it was hoped that a little longer time would give a more clear-cut demonstration of the possible differences between the effects of red and green light. Ovaries and oviducts of females killed at this time indicate that the order of stimulating power of light is red, white, green, for females. This is in agreement with the observed effects among males studied previous to this date.

13. On May 17, after 109 days of treatment, testes from the original lot (Table VIII) show that, while mature "green-light" birds have advanced a little since May 8, "red-light" birds of the same age are still at the climax of spermatogenesis, which they reached before May 8. Red light has maintained greater spermatogenic activity than green in these birds.

14. On this same date, after 60 days, "green-light" birds of the second lot (Table VIII) have continued to regress till they have reached almost, if not quite, the mid-winter condition. Green shows no power to induce progressive testis changes. No "red-light" birds were left for comparison.

15. In the cases of birds from the previous experiments carried over into this one, and starting these treatments at complete spermatogenesis, the results are in complete agreement with the effects noted in this study and in those found in the previous study (Bissonnette, 1931*b*). A "green-light" bird, killed on April 28 (Table VI), with light intensity reduced by about six-sevenths of its intensity, and in length of experimental period, at two different times, underwent regression twice, and reached almost mid-winter condition on that date.

TABLE VIII

BIRDS KILLED MAY 17, 1930

Cage	Age*	Gonad Size† (Mm.)	Color	Days in Cage	Remarks
Green light	M.	R. 4.0×2.6 L. 4.8×2.6	Dark	109	Fig. 10.—Appear to have progressed a little since May 8; nuclei in tubules more numerous; synizesis in few nuclei; nowhere near complete spermatogenesis.
Red light	M.	R. 12.7×9.1 L. 13.9×8.2	Light	109	Fig. 11.—Complete spermatogenesis; no change since May 8; maintained at this climax.
Green light	M.	R. 3.3×2.3 L. 3.7×1.9	Dark	60	Fig. 32.—Still regressing after May 8; débris cleared up and nuclei increasing in number; not maintained by green light but permitted to regress when daylight ration is cut down.

* M = mature bird; Y = juvenile bird.
† R = right testis; L = left testis.

Two "green-light" birds, also from the preceding experiment (60-watt cage), killed on May 8, after 100 days in this experiment, with even greater reduction in intensity of experimental treatments and the same two reductions in daily period, also underwent the double regression and failed to show any signs of renewed activity. Mature birds were more affected than young ones.

Two cases of abnormal testes, in birds covered by this study, also agree in manner of reaction to lights with the testes above described. They were cases of apparent non-pigmentation in testes which in normal cases are highly pigmented. One was the case of a young bird ("violet light") killed on March 29 (Plate III, Figs. 19, 20), in which both testes were white in color when all other testes of similar size are deep green and highly pigmented. On sectioning the left one, it was found to have a few small, widely scattered, pigment spots. This testis was also abnormal, in having a connective tissue core

with tubules ending near it with dilated ends as though they were blind. Germ-cell stages within the tubules were similar to those of other birds subject to similar treatment. Plumage pigmentation was apparently normal.

The other case is presented by a young "green-light" bird, killed on May 8 (Plate IV, Figs. 33, 34, 35, 36). In this bird, when killed, the left testis (Plate IV, Figs. 35, 36) was apparently unpigmented and white in color, while the right (Plate IV, Figs. 33, 34) was normally pigmented and dark green in color, and therefore comparable to testes of other birds studied. The left testis was found, on sectioning, to have very few small widely scattered pigment spots between a few tubules. This left the numerous interstitial cells for the most part unobscured by pigment (Plate IV, Fig. 35), while those of the normal testis (Plate IV, Fig. 33) are much obscured by it. Conditions within the tubules of the unpigmented testis are exactly like those in the pigmented one. Except for the pigmentation difference, the two testes are alike. Whether this is a case of somatic non-disjunction involving the chromosome carrying the gene-controlling pigment development, or some other type of asymmetry, is not yet understood. No differences in plumage pigmentation between different parts of the bird and the usual were noted, even on careful inspection.

One other bird, in our experience of over 6 years with starlings, had both testes apparently white in the mid-winter condition or near it. They also contained a few small pigment spots widely separated in the interstitial tissue between tubules.

In both series of birds beginning treatments on January 28 and March 18, starting from conditions prevailing in free birds outside, on those dates, red and white light stimulated progressive testis activity in general and green light was inhibitory in action and induced or aided regressive changes. In some cases, the induced changes were slight; and, in a few cases, red and white light seemed to fail to give the stimulating effect. These apparent exceptions may be due to a habit of roosting in darker parts of the cages where the light intensity is much less than the standard dose delivered at the center of the roost.

Seven hours of green light at the intensity used is unable to

compensate for the loss of $2\frac{1}{2}$ hours of daylight per day, in maintaining or accelerating testis activity. Red light is not able to do so at first, apparently; but later it enables testes to return to complete spermatogenic activity. White light appears to retard or prevent regression for a short time after the reduction in daylight period, but not later; and regression follows. This apparent anomaly is being further studied under more carefully controlled conditions.

Violet light, as used, does not stimulate to sexual activity, and in most cases retards it. However, the intensity used and the very slight responses noted make it impossible to state with any certainty its effects when used in the same intensity as the other lights.

A definite difference in the rate and degree of response of juvenile and mature birds to both progression- and regression-inducing light changes is apparent. Young birds respond more readily and completely to regression-inducing changes; mature ones to progression-inducing ones, in early stages, but not in later stages. Roosting habits may lead to considerable variation.

DISCUSSION

Many other authors have reported differences in biological effects of light from different regions of the solar spectrum, both from the visible and from the invisible (infra-red and ultra-violet) portions. It is not our purpose to discuss all the literature in this connection, but merely to show that this is by no means unusual. Papers pertaining to invisible rays will not be discussed.

Lights from Mazda and arc lamps in crown glass retard neoplastic growth (Pearce and Van Allen, 1927). Certain plants show weakest development when the incident light is chiefly red or blue, and intermediate development when violet and part of the blue are lacking. Both quality and intensity of light are factors in determining development in some plants (Pfeiffer, 1928).

Number of germinations of seeds is greatest under infra-red and ultra-violet parts of the solar spectrum, and least under green; weight and height of stalks are greatest in infra-red, red, violet, and ultra-violet regions, while chlorophyll develops best under green, and least under the extremes of ultra-violet and infra-red. Different types of radiant energy give different electrical potentials in plants

(Sheard, Higgins, and Foster, 1930*a*). These are in agreement with the differences for red and green as found by us for starling testis activity. As to violet-light action, we are unable to tell with any certainty what its action is in intensities comparable with those producing striking differences in reaction for red and green. It appeared to be inhibitory.

In chickens, constant ultra-violet radiation alone does not insure normal development; and constant long-waved visible light, alone, retards growth and development. But additions of very small amounts of ultra-violet to longer wave-lengths of visible light bring about normal growth and development. So both long wave-lengthed visible light and shorter ultra-violet radiations are necessary for complete and normal development and are complementary to each other in some respects (Sheard, Higgins, and Foster, 1930*b*).

For chlorophyll development in certain plants, with approximately equal energy values, red rays are more effective than green, and green than blue. The effectiveness of radiant energy appears to increase with wave-length to about 680 $\mu\mu$ and there ends abruptly (Sayre, 1928). Our experiments on starlings agree so far as red and green are concerned, but we cannot say as to violet light of the same intensity, and we used equal intensity of illumination and not equal total energy of irradiation. In further studies now under way both intensity of illumination and energy, as measured by a thermocouple, will be considered in relation to effects on testis activity.

Window glass and filters used in our experiments transmit little, if any, ultra-violet, and it can be left out of consideration here. However, it is interesting to note that little ultra-violet of physiological significance is to be found in sun's rays in winter in Chicago, because absorbed by smoke in the atmosphere, but that 300-watt incandescent lamps emit radiations, in the ultra-violet, of physiological interest (Bundesen, Lemon, Falk, and Conde, 1927).

Visible as well as ultra-violet rays in 15–30–60-minute doses cause hyperglycaemia in albino rats after a short latent period, which period is subject to modification by repeated treatments (Ceruti, 1930). These latent periods and their modifiability are of interest in the light of our findings of a latent or refractory period before onset of light-induced changes, prolonged by increase of period of muscu-

lar work (Bissonnette, 1931*a*, 1931*b*) and varying with the sort of light used, so far as white and red light are concerned.

That blood equilibrium changes probably accompany or mediate testis changes under light treatments with visible rays in these experiments is in accord with Brown's (1928) finding that long wave-length light of small energy content, and no ultra-violet, can produce definite changes in the chemical equilibrium of calcium and phosphorus in blood as well as affect growth, nutrition, and activity in rabbits. That this is correlated with parathyroid changes is probable in the light of recent findings that both visible and ultra-violet portions of radiant energy are necessary to normal parathyroid development (Higgins, Foster, and Sheard, 1930).

The fact that spring sunlight is relatively strongest and richest in red and infra-red; autumn sun, by comparison, richer in ultra-violet; and summer sunshine richest in the latter (Laurens, 1928), is suggestive in connection with our finding that red is most effective in stimulating spermatogenesis. The longer red and infra-red rays are believed to penetrate deeper than those toward the other end of the spectrum. Luminous rays, particularly the red, can pass through several centimeters of the human body (Laurens, 1928). It seems hardly possible that the testes of these starlings may be reached directly by the red rays through the body and almost black feathers. But, if so, the greater effect of red rays than green might be partly accounted for. Then, too, differences between the biological effects of rays from different parts of the spectrum, even close together, as found by many, are in accord with such differences in effects upon the testes of these birds.

Greater penetration power of red than green light may account, in part, for the greater effects of red, and for the stimulating effect of white light, which contains some red rays; for, in many cases, penetration power has been shown to increase with wave-length. For the differences in penetrability of face and chest skins in human beings, for instance, this holds. Then, too, shorter rays are more completely absorbed by epidermis, by colorless and yellow scales, and by the webs of frogs' feet with the blood flowing through them (Laurens, 1928). These parts of the coverings of birds are the ones most likely to be receptive for the light, and these findings are particularly

pertinent to the question as to how the rays attain their initial effect upon the birds.

On the other hand, in *Amoeba proteus*, the absorption curve of protoplasm is low for wave-lengths longer than 320 $\mu\mu$, rises abruptly to a peak at 280 $\mu\mu$, descends as abruptly to half its height, with a minimum at 250 $\mu\mu$, and rises again with extreme abruptness to very high values in the shorter wave-lengths. The absorption coefficient varies from 200, at 238 $\mu\mu$ to 1,000, at 200 $\mu\mu$, showing that the amount absorbed from different regions of the spectrum varies irregularly and not in direct proportion to wave-length (Laurens, 1928). The Lambert-Beer law, that the fraction absorbed by a given thickness of protoplasm is independent of the intensity of light, has been shown to hold for physiological effects of radiation, and also the Grotthus-Draper law, that only absorbed radiation has any effect (Collier, 1931). It is not impossible that the germinal epithelium of starling testes may show selective absorption for certain wave-lengths of the spectrum, if these wave-lengths can reach them. The dark-green pigment between tubules in small-sized testes should prevent all visible rays but green from passing through to the germinal epithelium, as well as reflect some of the green. It may well act as an absorption screen, and in all these ways modify the effects of light.

For human skin, the amounts of visible, inner and outer infra-red, that can be borne, are as 3.11:1.79:1.33, respectively, while amounts absorbed are as 2.02:1.16:1.33 in gram calories per square centimeter per minute. For the highest endurable intensity of visible energy, the skin temperature rises to 43.8° C.; with outer infra-red, to 45.5° C.; but, 15 seconds after irradiation, the relation is reversed; so that skin temperature after visible rays is 1° C. the higher. So subcutaneous temperature is the greater during irradiation with the visible wave-lengths. For visible rays the maximum temperature at 0.5 cm. depth is 47.5° C., while that for outer infra-red is 41.7° C., and at 1.2 cm. depth the temperatures are identical at 44.6° C. (Sonne, 1927). These depths are not beyond the range of depth of the starling testis below the surface of the back, so these findings may be significant for the effects noted on testis tissue, if the penetration is between the ruffled feathers upon the skin directly, and the

birds agree with man so far as absorption and temperature effects are concerned.

It is suggested by Sonne (1927) that heating of subcutaneous tissues and blood therein, by the visible part of the heat spectrum, to temperatures above those experienced in fevers, without general rise in body temperature, owing to heat regulation in the body, is the main feature of the action of radiant energy (Laurens, 1928). Deeper and denser material than is commonly supposed is penetrable by intense enough light and long exposure (Mayer, 1926). Thus both time and intensity of illumination are factors in the biological effects of radiation. This agrees with our previous findings (Bissonnette, 1930*a*, 1931*a*, 1931*b*), and, taken with the greater penetration power of longer-waved light, and its greater absorption at deeper levels in higher animals, points to the probability that it is greater absorption at deeper levels that may give red light its greater effectiveness than green, in inducing rapid multiplication of germ-cells in starling testes. But it would hardly account for the inhibitory action of green. So, direct effect upon the germinal epithelium of these testes would hardly appear probable.

It has been pointed out that visible and near infra-red rays with considerable penetration power stimulate epithelization in wound-healing by acting directly on mitoses (Laurens, 1928). Red is more effective in inducing mitoses in starling testes than green.

In connection with the question as to what part of the body is receptive to the light rays in birds, it has been determined by Goodale (1926) that the natural coverings of chicks are not penetrated by wave-lengths of 208–312 $\mu\mu$ which are those conditioning normal growth, and that the main receptor is the small amount of naked skin about the head. In this finding he is supported by Domm's suggestion (1930). However, Hou (1931) has shown that, with preen gland intact, feet and legs, head, and dorsum of the body and wings, all can absorb enough ultra-violet to correct rickets, but only the feet and legs, in the absence of the gland and its oil over the body. This has not yet been tested for starlings for wave-lengths that produce these testis effects, under controlled conditions.

Exogenous metabolic processes in plants are influenced by the longer wave-lengths of light (Sheard and Higgins, 1927).

Increase in oxygen consumption in meal-worm chrysalids varies with the sort of light used to irradiate them, but yellow light has no influence on gaseous metabolism. There is a persistence of increased metabolic rate if unfiltered light is used, but not if ultra-violet is filtered out (Sonne, 1927). This suggests that altered metabolism is at the root of the different effects of light rays from different spectral regions and of persistence of any degree of activity after light changes, which we have called the "lag" or "refractory" period.

On the contrary, in the frog, which responds to increased light with increased respiratory movement, the increase bears no quantitative relation to the wave-length of light, and the transitory change disappears in 2 minutes (Croft and Laurens, 1924).

In general, rays with wave-lengths longer than 290 $\mu\mu$ are stimulating; shorter ones are lethal in effect. Effects produced are photochemical, leading to dermatitis and its logical sequelae on the one hand, and biological, lasting for some time on the other. The effects studied by us were of the latter sort, but green was inhibiting in effect.

Part of the difference in effect of green and red light may be due to reflection of green rays by the green pigment between and around the tubules, reducing the absorption from green light there. Since ovaries lack this pigment, this difference in effect, so caused, should not be so great in them as in testes, if pigment is a factor in the difference. Female material from all these experiments is now awaiting study.

That there is an optimum time for added illumination for laying hens has been shown (Peter, 1929), together with the fact that no ill effects upon fertility and vitality, necessary to reach the hatching stage, follow such irradiation of hens. So, illumination with electric light after dark, principally for laying hens, has been common practice among poultrymen seeking high egg production.

Recently it has been found that irradiation of ergosterol with red light, after activation with ultra-violet, destroys its anti-rachitic properties. Irradiation of rats, fed activated ergosterol, with red light caused them to develop rickets, whereas controls fed the same amounts of activated ergosterol but not treated with red light did not. Red light is therefore antagonistic to ultra-violet so far as prevention

of rickets is concerned (Ludwig and von Ries, 1929). It is therefore not surprising that red and green light are opposite in effect upon the germinal epithelium, red being stimulating and green slightly inhibiting. In this connection it is just possible that part of the cause of the regression of testis activity in early June in nature may be the excess of green light reaching the birds by reflection from foliage and grass during the day, and its gradual preponderance over the red rays of spring sunlight. Should that be so, it is not difficult to understand the holding-back of sexual activity throughout summer and early autumn at least till the reds and yellows of autumn tints appear in the leaves, at which time the lessening effective intensity and period of daylight in autumn and late summer would prove effective in inhibiting sexual activity in these birds. The possible participation of the increasing ultra-violet of summer sunshine in this early regression is being investigated.

The studies above discussed in relation to our results show that there is a difference in biological effect between rays of visible light from the solar and electric-light spectra depending on the wave-length, and that spermatogenic activity is one of the processes showing variations in relation to type of illumination as well as intensity and duration. They are in accord with the generalization that longer wave-lengths of light are stimulating in effect and shorter ones inhibitory.

Theories as to the mode of action of the rays upon protoplasm have been discussed in a previous paper (Bissonnette, 1931*b*), and will not be gone into here; nor will the relation of pigment to this action and to penetration depth, nor the influence of differential penetration, rather than action specific for wave-length.

SUMMARY AND CONCLUSIONS

1. Studies were made of the effects upon spermatogenic activity, in starlings, of equal intensities of illumination for equal periods, of unfiltered light, and filtered red and green lights from incandescent bulbs, at about 2.6 foot-candles, and of filtered violet light at about 0.32 foot-candles, with controls.

2. Experimental light doses increased by half-hour additions up to 7 hours per night. Up to March 5, these doses were added to

normally increasing daylight periods. Thereafter, the constant 7-hour dose was added to a constant $9\frac{3}{4}$-hour daylight period ($1\frac{3}{4}$ hours reduction of daylight on that date). Samples were killed for gonad study at 19, 33, 49, 60, 74, 90, 100, and 109 days.

3. A second lot of birds was introduced from outside on March 18, and given 7 hours of artificial light, added to $9\frac{3}{4}$ hours of daily daylight (reduction of $2\frac{1}{2}$ hours of daylight per day). Samples were killed for gonad study at 11, 25, 41, 51, and 60 days, to test the power of these artificial light doses to prevent regression due to decrease of the daily daylight from March 18 onward.

4. A few birds, brought to complete spermatogenesis by various intensities of light during previous experiments, were carried over into these experiments to test the power of these smaller light doses to maintain them at complete activity or to retard regressive changes due to reduced light ration.

5. Testes of mature birds (having passed one season of sexual activity) consistently react more quickly to spermatogenesis-inducing light changes, in early stages of treatment, than do juvenile ones (still in their first year); juvenile ones react more quickly to regression-inducing light changes and more completely to later stages of activating ones.

6. In early days of treatment, white light appears to give greatest acceleration of testis activity, red next, and green, a slight inhibitory effect; but, by 33 days, red-lighted birds have surpassed those receiving white light, which are slightly more active than controls, and green-lighted birds are definitely inhibited.

7. "Red-light" birds reached complete spermatogenesis, but no "white" or "green-light" ones did, prior to the reduction of light rations. However, white light retarded regression of birds already at, or very near, complete spermatogenesis, in early stages of treatment; red did not; and green hastened it.

8. On reduction of daylight rations, white-light treatments, in early stages, best prevented regression; red-light, in later states. "Red-light" birds were best maintained in activity in longer continued treatments. All birds underwent some regression, following reduction of daylight, but it was delayed in "white-light" birds more than in "red-light" ones.

9. A refractory period intervened between the time of change in light ration and the appearance of testis changes, in either direction. This varied with the type of treatment given.

10. After decrease of light rations, all birds came to a state of quiescence at or above the minimal mid-winter condition, and, with some types of experimental light dose, resumed progressive changes. Some birds were maintained at a state of partial regression for a considerable time, with constant light rations. None were maintained at maximum activity or complete spermatogenesis for longer than 30 days. Mature birds resisted regression better than young ones.

11. Birds were induced to undergo regression when well on the way to maximum activity, and were then maintained in a state of partial regression or in the mid-winter condition, through the normal breeding season, even when their total daily light periods exceeded the length of day in the normal breeding season.

12. In common with many other biological processes in plants and animals, seasonal sexual activity in starlings is susceptible to modification and is conditioned by wave-length of light as well as its intensity and length of daily period.

13. The possibility of sensitizing or supplementary effects of different wave-lengths of radiation contained in white light, the relation of intertubular pigment to differences in effects of different wave-lengths, tissue temperatures at different depths in relation to penetration power of visible rays, with possible relation to mitosis, and the probable antagonism of longer wave-lengths for shorter ones are discussed.

14. It is suggested that, in nature, the progressive testis changes in spring may be helped by the relative strength of red rays in the sunlight of that season, and the regression beginning before June 21, in nature, may be due to the relative excess of inhibitory or lethal green (and perhaps violet and ultra-violet) rays in summer and early autumn. Green reflected from foliage and grass may help this. Regression from complete spermatogenesis may also be due to an inability to maintain this activity for more than a certain time, even under conditions optimum for inducing increase of activity at other stages.

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EXPLANATION OF PLATES

PLATE I

All figures are photomicrographs of comparable tubule sections taken in the first instance at 335 diameters and reduced to 223 diameters in printing. Type sections are given where several sections are so much alike as to show no differences appreciable at the reduced magnification. Description of the bird from which the figure is taken is given in italics, other similar sections come from birds described but not in italics.

FIG. 1.—Bird No. 1. Young "inside control," confined January 28—February 15, 19 days.

Bird No. 6. Mature "red-light" bird, confined January 28—February 15, 19 days.

Bird No. 7. Young "red-light" bird, treated January 28—February 15, 19 days.

Bird No. 9. Young "green-light" bird, treated January 28—February 15, 19 days.

Bird No. 20. Mature "inside control," confined January 28—March 17, 49 days.

Bird No. 59. Young "green-light" bird, treated March 18—April 28, 41 days.

FIG. 2.—*Bird No. 2. Mature "inside control," confined January 28—February 15, 19 days.*

Bird No. 17. Mature "green-light" bird, treated January 28—March 1, 33 days.

Bird No. 21. Mature "violet-light" bird, treated March 5–17, 37 days as control plus 12 days of violet light.

Bird No. 22. Young "violet-light" bird, treated as above, 37 days as control plus 12 days of violet light.

Bird No. 32. Mature "violet-light" bird, treated March 5–29, 23 days of violet light.

Bird No. 64. Young "green-light" bird, treated January 28—May 8, 100 days.

FIG. 3.—*Bird No. 3. Young "white-light" bird, treated January 28—February 15, 19 days.*

Fig. 4.—*Bird No. 4. Mature "white-light" bird, treated January 28—February 15, 19 days.*

Bird No. 14. Mature "white-light" bird, treated January 28—March 1, 33 days.

Bird No. 34. Mature "green-light" bird, treated January 28—March 29, 60 days.

Bird No. 19. Mature "inside control," confined January 28—March 17, 49 days.

FIG. 5.—Bird No. 5. Young bird treated with 15-watt bulb (131 lumens) at 9 inches distance, November 9—January 27. At complete spermatogenesis on that date. Treated with same white light at 24 inches distance until February 15, 79 plus 19 days.

Bird No. 27. Mature "red-light" bird, treated January 28—March 17, 49 days.

Bird No. 61. Young "white-light" bird, treated March 18—April 28, 41 days.

FIG. 6.—Bird No. 8. Mature "green-light" bird, treated January 28—February 15, 19 days.

Bird No. 33. Young "green-light" bird, treated January 28—March 29, 60 days.

Bird No. 44. Young "inside control," confined January 28—April 12, 74 days.

FIG. 7.—*Bird No. 10. Young "inside control," confined January 28—March 1, 33 days.*

FIG. 8.—Bird No. 11. Mature "inside control," confined January 28—March 1, 33 days.

Bird No. 26. Mature "white-light" bird, treated January 28—March 17, 49 days.

Bird No. 45. Young "green-light" bird, treated January 28—April 12, 74 days.

Bird No. 43. Young "inside control," confined January 28—April 12, 74 days.

Bird No. 51. Mature "red-light" bird, treated March 18—April 12, 25 days.

Fig. 9.—*Bird No. 12. Mature "white-light" bird, treated with 15-watt bulb* at 9 inches distance November 9—January 27 and at 25 inches distance January 28—March 1; 79 days at 9 inches plus 33 days at 24 inches. (Was at complete spermatogenesis on January 28, when intensity of illumination was reduced by increase of distance.)

PLATE II

FIG. 10.—Bird No. 13. Young "white-light" bird, treated January 28—March 1, 33 days.

Bird No. 76. Mature "green-light" bird, treated January 28—May 17, 109 days.

FIG. 11.—Bird No. 15. Young "red-light" bird, treated January 28—March 1, 33 days.

Bird No. 48. Young "red-light" bird, treated January 28—April 12, 74 days.

Bird No. 57. Young "red-light" bird, treated January 28—April 28, 90 days.

Bird No. 69. Mature "red-light" bird, treated January 28—May 8, 100 days.

Bird No. 77. Mature "red-light" bird, treated January 28—May 17, 109 days.

FIG. 12.—Bird No. 16. Mature "red-light" bird, treated January 28—March 1, 33 days.

Bird No. 35. Mature "red-light" bird, treated January 28—March 29, 60 days.

Bird No. 66. Young "green-light" bird, treated with green January 28—May 8, after 60 watts of white light November 9—January 27, 79 days. Strong white to complete spermatogenesis plus 100 days of green.

FIG. 13.—*Bird No. 18. Young "green-light" bird, treated January 28—March 1, 33 days.*

FIG. 14.—Bird No. 46. Mature "green-light" bird, treated January 28—April 12, 74 days.

Bird No. 49. Mature "green-light" bird, treated March 18—April 12, 25 days.

Bird No. 56. Mature "green-light" bird, treated January 28—April 28, 90 days.

FIG. 15.—*Bird No. 23. Young "green-light" bird, treated January 28—March 17, 49 days.*

FIG. 16.—Bird No. 24. Mature "green-light" bird, treated January 28—March 17, 49 days.

Bird No. 25. Mature "white-light" bird, treated January 28—March 17, 49 days.

Bird No. 28. Young "red-light" bird, treated January 28—March 17, 49 days.

Bird No. 52. Young "white-light" bird, treated March 18—April 12, 25 days.

Bird No. 71. Mature "green-light" bird, treated March 18—May 8, 51 days.

FIG. 17.—*Bird No. 29. Mature "red-light" bird, treated with 25-watt bulb from November 9 to January 27 at 9 inches distance, then with red light until he died on February 4; 79 days of strong white light plus 7 days of weaker red.*

Bird No. 53. Mature "white-light" bird, treated March 18—April 12, 25 days.

Bird No. 37. Mature "green-light" bird, treated March 18–29, 11 days.

Bird No. 38. Young "green-light" bird, treated March 18–29, 11 days.

FIG. 18.—*Bird No. 30. Mature "inside control," confined January 28—March 29, 60 days.*

Bird No. 36. Young "red-light" bird, treated January 28—March 29, 60 days.

PLATE III

FIG. 19.—*Bird No. 31. Young "violet-light" bird, treated March 5–29,* after being confined as "inside control" from January 28, 37 days as control plus 23 as violet-light-treated bird. Both testes of this bird were almost without pigment.

FIG. 20.—*Bird No. 31. Low-power photomicrograph of same testis as Figure 19* (*79*) to show lack of pigment except for few small flakes.

PLATE I

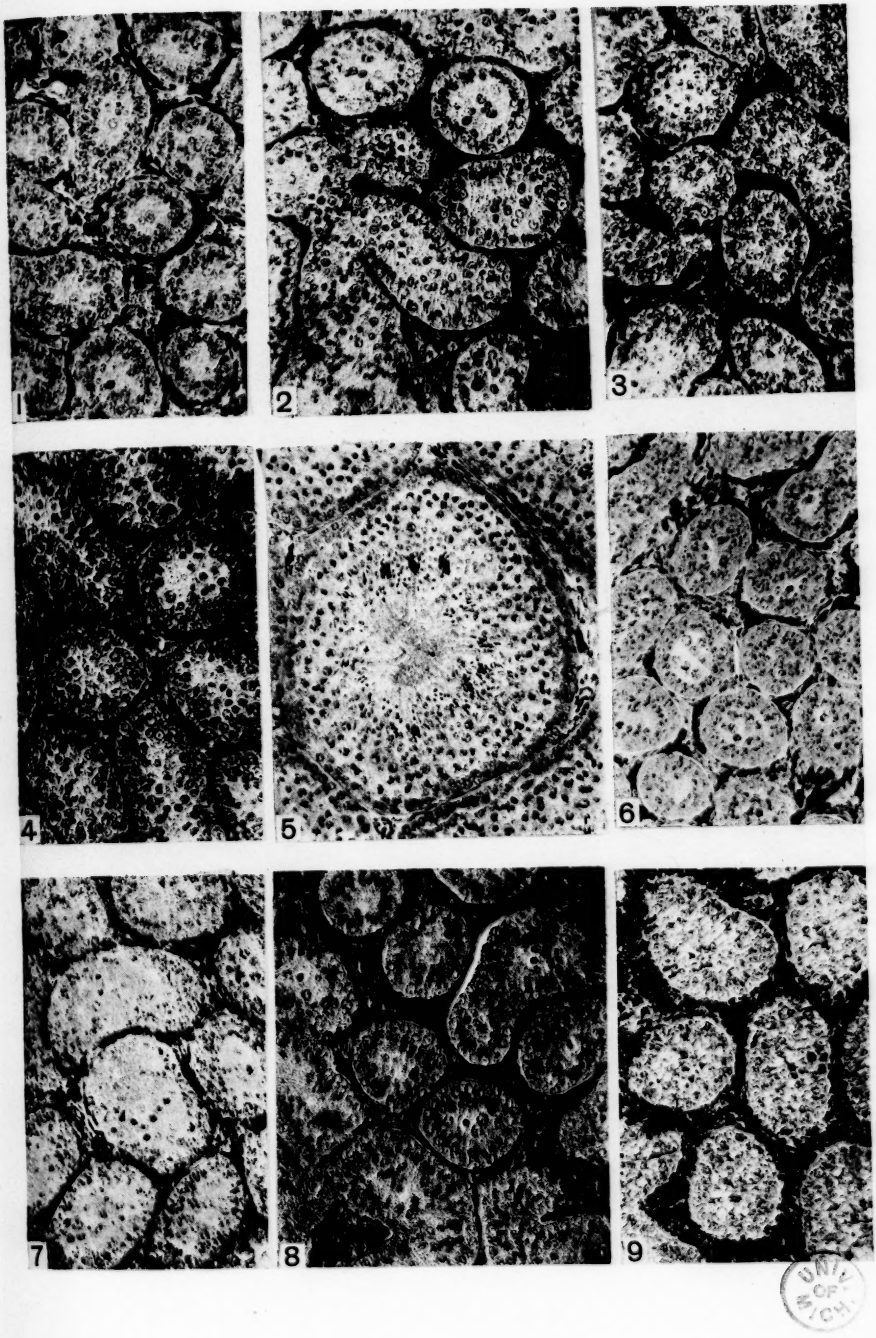

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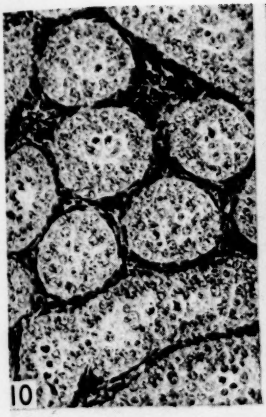
PLATE II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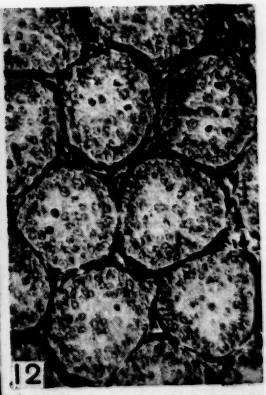
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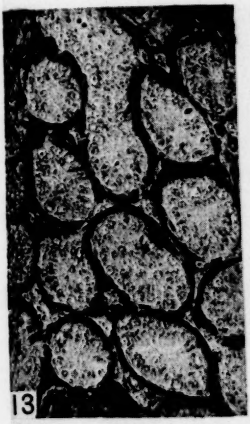
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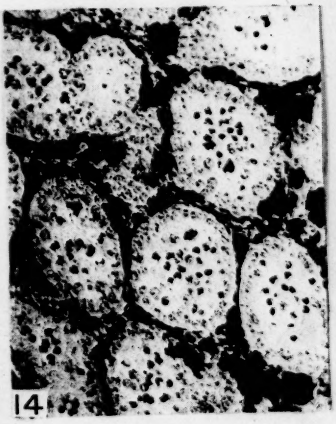
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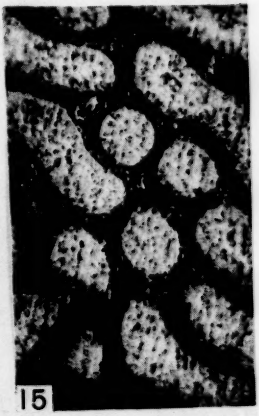
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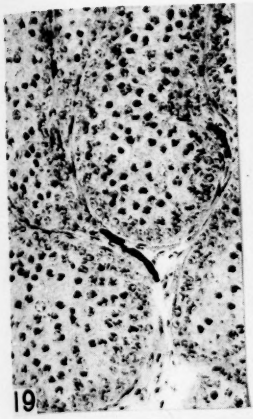
PLATE III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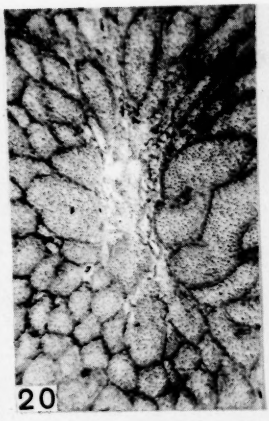
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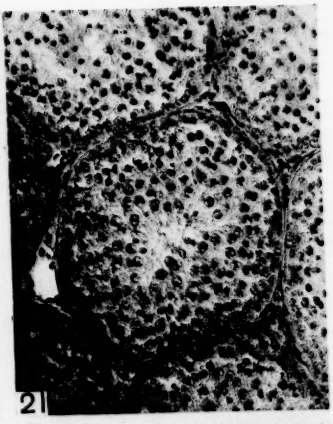
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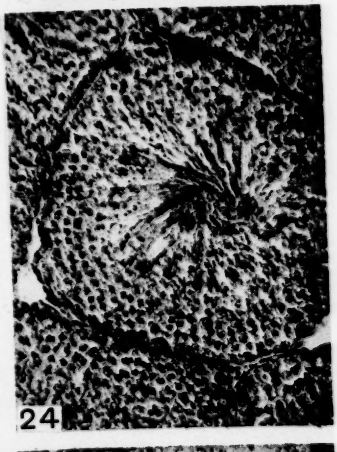
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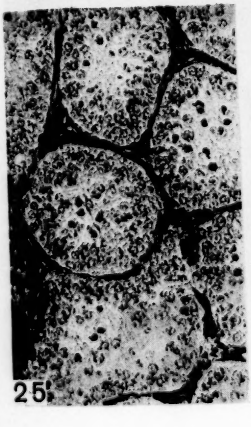
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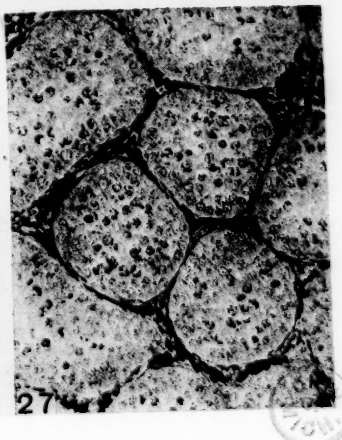
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PLATE IV

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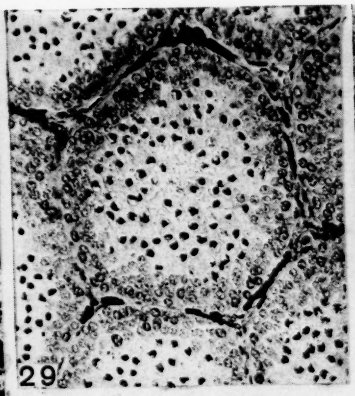
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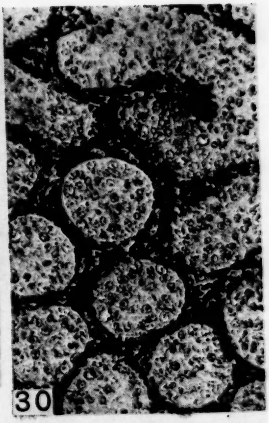
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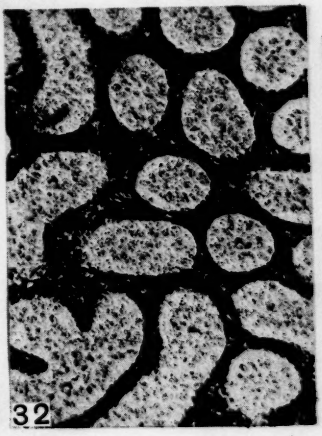
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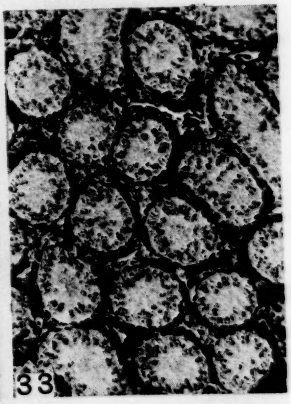
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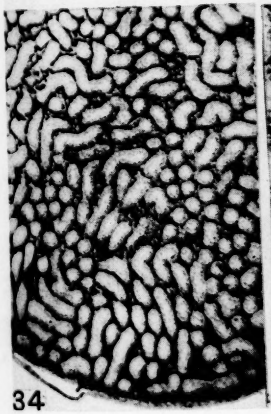
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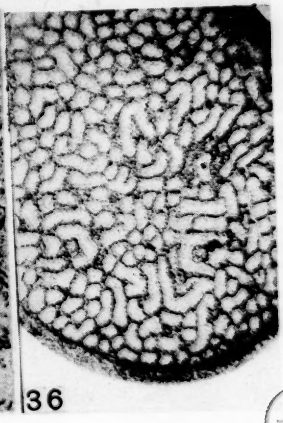
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FIG. 21.—*Bird No. 47. Mature "red-light" bird, treated January 28—April 12, 74 days.*

FIG. 22.—*Bird No. 39. Mature "red-light" bird, treated March 18–29, 11 days.*

FIG. 23.—Bird No. 40. Young "red-light" bird, treated March 18–29, 11 days.

Bird No. 55. Mature "green-light" bird, treated January 28—April 28, 90 days.

Bird No. 62. Mature "white-light" bird, treated March 18—April 28, 41 days.

FIG. 24.—*Bird No. 41. Mature "white-light" bird, treated March 18–29, 11 days.*

FIG. 25.—*Bird No. 42. Young "white-light" bird, treated March 18–29, 11 days.*

FIG. 26.—*Bird No. 50. Young "red-light" bird, treated March 18—April 12, 25 days.*

FIG. 27.—*Bird No. 63. Mature "red-light" bird, treated March 18—April 28, 41 days.*

PLATE IV

FIG. 28.—Bird No. 54. Young "green-light" bird, treated with green, January 28—April 28, after being brought to complete spermatogenesis on January 27, by 15-watt bulb at 9 inches distance from November 9 to January 27; 79 days at white light plus 90 days of green.

Bird No. 68. Young "red-light" bird, treated January 28—May 8, 100 days.

FIG. 29.—*Bird No. 58. Mature "red-light" bird, treated January 28—April 28, 90 days.*

FIG. 30.—Bird No. 65. Mature "green-light" bird, treated January 28—May 8, 100 days.

Bird No. 70. Mature "white-light" bird, treated March 18—May 8, 51 days.

FIG. 31.—*Bird No. 60. Mature "green-light" bird, treated March 18—April 28, 41 days.*

FIG. 32.—*Bird No. 67. Mature "green-light" bird, treated January 28—May 8, 100 days,* after 79 days from 60-watt bulb of white light at 9 inches from November 9 to January 27.

Bird No. 78. Mature "green-light" bird, treated March 18—May 17, 60 days.

FIG. 33.—*Bird No. 72. Right testis (pigmented) of young "green-light" bird, treated March 18—May 8, 51 days.*

FIG. 34.—*Bird No. 72. Low-power photomicrograph of same testis as Figure 33,* showing normal pigment distribution for testes of such stage.

FIG. 35.—*Bird No. 72. Left testis (almost unpigmented) of same bird as Figure 33, to same magnification (335 reduced to 223).*

FIG. 36.—*Bird No. 72.—Low-power photomicrograph of same testis as Figure 35, to show almost total lack of pigmentation, for comparison with Figure 34, of right testis from same bird.*

THE PHYSIOLOGY OF DEVELOPMENT OF FEATHERS. I. GROWTH-RATE AND PATTERN IN THE INDIVIDUAL FEATHER[1]

(Eight plates and thirty figures)

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University of Chicago

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[1] The expenses of this investigation were supported in part by the Committee for Research in Problems of Sex of the National Research Council.

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I. INTRODUCTION

The studies of the last eight years in the Whitman Laboratory of Experimental Zoölogy of the University of Chicago on the biology of sex in fowl have impressed us with the lability of the form and pattern of feathers, which render them an admirable index of the physiological condition of the bird, not only with reference to the female sex hormone (oestrin), but also to thyroxin and certain conditions of nutrition. The studies of Juhn and Gustavson (1930), Juhn, Faulkner, and Gustavson (1931), and Juhn and Barnes (1931) show how feathers may be used as indicators. Domm's earlier studies (1927) had previously shown that the feathers reflect in a very accurate way the gonadic condition of the bird, however modified experimentally.

These results led naturally to an attempt to analyze the physiology of development of the individual feather, in order to see how the effects are produced.[1] The nearest approach so far in our previous studies to this theme is the discovery by Juhn and Gustavson (1930) that the threshold of reaction of different feathers of the male and capon to the female sex hormone is directly proportional to the rates of growth along their axes. Different feather tracts have different rates of growth and correspondingly react at different levels of concentration of the female hormone, thus producing controllable mosaic gynandromorph conditions so far as plumage is concerned. Zawadowsky (1926) had previously noted a difference of threshold of reaction to the female hormone in different feather tracts; but the subject first received a quantitative treatment and interpreta-

[1] We are greatly indebted to Professor R. G. Gustavson for the preparation and standardization of the female hormone used in the experiments. The source in all cases was human pregnancy urine.

tion in terms of rates of growth by Juhn, Faulkner, and Gustavson (1931).

The present study concerns the individual feather primarily, the means of modifying its normal pattern and the mechanism of development by which such results are attained. The feather is an admirable system for studies in the physiology of development, on account of its symmetry relations, its relative simplicity of structure, its availability at all times, and its precise response to physiological conditions that can be controlled quantitatively.

As in all developmental systems, the range of physiological control is limited by the genetic organization of the species and breed (cf. Danforth and Foster, 1929). But a second limiting factor, equally decisive, also exists in the fowl in the qualities of the various feather tracts, which constitute a mosaic of self-determining feather types (Domm—in press). It thus becomes necessary to specify not only the species and breed dealt with but also the feather tract, and in some cases even the part of the tract. Such precisely determined observations are the only safe basis for generalization.

The present paper is limited to the Brown Leghorn fowl, which we have studied for 8 years with the intention of providing a base from which other genetic compositions can be attacked.

We shall begin with a statement of principles and with diagrams, for which evidence will be presented in the descriptive and experimental parts of the work. By so doing, the reasons for the facts and experiments selected for presentation will be clear from the outset; and also the reasons why certain descriptive matters, which might be expanded almost indefinitely, are omitted or barely mentioned.

The feather is the most perfect example of the principle of concrescence in the origin of a bilateral system that we know. The two halves arise from a ring of embryonic cells, hereafter designated as the "collar," which surrounds the base of the pulp of the feather germ. This ring, like a signet ring, is bilaterally symmetrical. The radii, or barbs, of the future feather arise from the collar at right angles to it, thus parallel to the axis of the feather germ. The shaft of the feather, or rhachis, is formed by a process of concrescence of the continuously growing right and left halves of the ring, the levels from apex to base being formed successively. The forming barbs are

carried along with the collar. There is thus a continuous process of migration of the forming barbs with the constantly streaming halves of the ring to their definitive positions at the sides of the shaft, with consequent change of orientation. The region of concrescence will be called the "dorsal surface" of the ring; and the region opposite (or approximately opposite), the "ventral surface." These do not correspond to the future outer and inner surfaces of the shaft, often called "dorsal" and "ventral," but to different transverse levels of the future shaft.

The order of formation of the shaft of the feather is strictly apico-basal. The order of age of the barbs is naturally the same. Similarly in each barb the apex at the margin of the feather is the first formed, and the central end attached to the shaft last. Thus there are two *time* gradients in each feather, viz.: from apex to base along the shaft, and from margin to center along the barbs.

At any given time, after the development of the feather is well begun, the collar is thickly beset with a series of forming barbs on each side (Plate I, Fig. 3), which range in age from the dorsal to the ventral surface of the feather germ; and in state of development from the completely formed barb dorsally to more apical rudiments ventrally. As the series of barbs move dorsally with the streaming halves of the ring, new barbs take their origin in the ventral space thus provided.

All of this may be represented in a single diagram (text Fig. 1). This represents the right half of a "split preparation" of a feather germ of the breast 12 days old (Plate III, Fig. 15), pulp removed and spread out flat. The cut edge represented at *V* is the mid-ventral line of the collar. In the diagram the horizontal diameter is magnified four times as much as the vertical diameter. The spiral course of the barbs is straightened out. The numbers 21, 25, 29, etc., at the apices of the barbs are the approximate serial numbers counting from the tip of the feather, about twenty barbs being already formed and attached to the rhachis. The numbers 10, 9, 8, 7, etc., at the bottom represent days of growth from time of origin in the mid-ventral line. Thus the barb numbered 61 will in 10 more days be in the position 10 below. The line uniting the apices of the barbs thus forms a curve of growth of an individual barb from origin to com-

pletion, assuming, as is actually the case in the breast feather of the fowl, that the series of barbs concerned are all approximately of the same length. (See legend of figure for additional explanation.)

The processes of differentiation begin in each barb a short distance above its place of origin in the collar (text Fig. 1, *Z*); hence the reactive region, or place of determination, of each barb is confined to the region immediately adjacent to the collar (text Fig. 1). It therefore follows that in the series of forming barbs all regions from center to margin of vane are simultaneously represented by reactive regions, and hence may be simultaneously influenced. The form and pattern of the feather are expressed primarily in the barbs and their attached barbules, though it is obvious that the shaft, as basis of attachment of the barbs, may also play an important rôle.

The rate of growth of the shaft is approximately uniform throughout its length, during the formation of the vane of the feather at least. The rate of growth of the barbs, on the other hand, diminishes from the apex to the base of each, that is, from the margin of the feather to the shaft. The form of the curve of growth representing this gradient plays a large part in determining the pigmentation and general pattern of the feather. This depends on the fact that each morphogenetic event that we study has a threshold of reaction which varies with the rate of growth of the affected system, being relatively low when the rate of growth is low, and vice versa. A continuously acting condition of low intensity may thus affect only the more slowly growing parts of the barbs, i.e., the reaction zone near the rhachis; but, if it be of an intensity above the threshold of reaction of the most rapidly growing regions, the entire extent of the reaction zone will be affected, and so the entire vane. If the condition acts discontinuously, as in an interrupted series of hormone injections, the principle of threshold differences operates in a more complicated manner.

Now it will be noted that the zone of reaction, *Z*, occupies, in the series of barbs from youngest to oldest, positions successively nearer the rhachis, starting from the ventral surface, *V*, of the collar. In the definitive feather, these positions correspond to a line at right angles to the rhachis owing to the obliquity of the axis of the

barbs to the rhachis, as the experiments to be described demonstrate. Thus, any condition that affects the zone of determination simultaneously throughout its entire extent will form a transverse

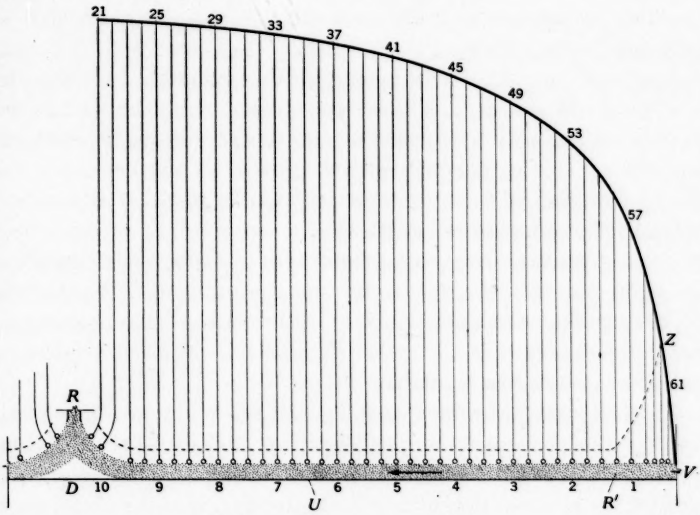


FIG. 1.—Brown Leghorn fowl. Diagram of organization of 12-day feather germ from the breast. The base of the preparation is magnified four times more than the height. The diagram represents the right half of a feather germ which has been split in the mid-ventral line, pulp removed and spread out flat. The rhachis has been cut across at *R*, and removed with its 20 attached barbs on each side. Each vertical line represents a forming barb. The spiral course of the barbs has been straightened out. The numbers 21, 25, 29, etc., at the apices of the barbs are the serial numbers from the apex of the feather. The numbers 10, 9, 8, 7, etc., at the bottom represent days of growth from time of origin in the mid-ventral line. The barbs are represented as evenly spaced except for the first day, although the distances apart along the collar increase slightly from ventral to dorsal. The heavy line uniting the apices of the barbs is taken to represent the curve of growth-rate of any individual barb (see text). It is not intended to be more than approximately correct. *D* marks the dorsal surface of the collar; *V*, the ventral surface. *R-R'*, stippled, represents the part of the collar concerned in formation of the rhachis. *Z*, broken line, marks the boundary between the zone of determination (morphogenetic reactions) on its basal side and the zone of differentiation. The arrow represents the direction of the constant streaming movement in the primordium of the rhachis. The circles at the base of the barbs represent the position of the centers of growth of the barbs. The barb centers and rhachis primordium combined constitute the collar. *U* = lip of umbilicus.

bar in the definitive feather, of width dependent on duration of the condition. But a condition of submaximal intensity affects only the more slowly growing barbs (those nearer to the rhachis) to an extent dependent on its value. The *principle of threshold of reaction* thus operates to produce patterns centering on the rhachis, which we may call "axial patterns."

Feathers are not strictly symmetrical bilaterally. In many this is at once obvious in their gross appearance, the vane on one side of the rhachis being narrower than on the opposite side. Such differences are due to unequal rates of growth on the two sides, and are often accompanied by differences of color or other elements of pattern. The principle of threshold of reaction comes into play here, and one of the most interesting features of our experiments has been the light that this principle throws on the problem of asymmetry, which explains certain anomalous phenomena. The experiments make it probable that all feather germs have an initial asymmetry which is more or less regulable.

Another principle, which we may call the "principle of reaction rate," is also of great theoretical significance. This is, that the time required for realization of a reaction is proportional to rate of growth; thus a condition of maximal intensity, if of very brief duration, will be recorded only in the most rapidly growing region, corresponding to the margin of the feather. This is the only principle that can explain patterns of positive *marginal origin*. Other marginal patterns are negative results of incomplete bars, diamond-shaped markings, or triangles of axial origin.

From a flowing curve of growth, such as shown in text Figure 1, we can explain only patterns that have a transverse method of extension. These may, of course, produce longitudinal patterns centering on the axis if the determining condition is submaximal and constant. But, in order to explain longitudinal patterns in the vane between axis and margin, as in "striping" or "penciling" (e.g., dark Brahma breed of fowls), it is necessary to assume, not a flowing curve of growth, but one with definitely placed irregularities, either of depression or elevation. Any such irregularity occupying a definite place between rhachis and margin would, under proper threshold conditions, record a continuous longitudinal stripe. This is,

however, purely theoretical and is not dealt with in the body of the paper.

It would seem possible by combination of these principles theoretically to explain many forms of feather pattern. But we do not propose to enter into this subject in the present paper, and we shall confine ourselves in what follows to the actual observations and experiments and their interpretation.

II. THE DEVELOPMENT OF THE FEATHER

A. GENERAL

In order to analyze the experimental modifications dealt with in Part III, it has been necessary to make a new study of the development of the feather; for the previous studies of the subject, which in any comprehensive sense are rather old, leave fundamental gaps, which must be filled in for the form of analysis that we propose. The beautiful study of Davies (1889), for instance, was dominated by the phylogenetic point of view, and was thus concerned with establishing the basis for a strict comparison with the reptilian scale on the one hand and the mammalian hair on the other, rather than with an interpretation of existing form and pattern. It nevertheless presents so critical and accurate an examination of previous research, and so thorough an analysis of certain main features of development, that it has quite generally been regarded as a definitive treatment of the subject. Strong's excellent study (1902) of the histogenesis of barbs, barbules, and especially of pigmentation is also a very important contribution to our knowledge of the development of the feather.

We shall attempt to supplement the results of previous authors on the following points:

1. The seriation of the barbs in development.
2. The rate of formation of barbs; number arising per day; time required to complete a barb.
3. The disto-proximal order of formation and differentiation of the individual barb.
4. The problem of a growth gradient in barbs.
5. The relation of the shaft to the barbs during development.
6. The problem of asymmetry of the feather.

7. The localization of determination of the morphological elements and pigmentation of the barbs and shaft with reference to time and place in the feather germ—in short, the projection of the feather back upon the germ. The consideration of these subjects will be distributed between Parts II and III.

Points 1 and 3, and to some extent also 5, have been considered by previous authors. The other questions have not. They are, however, precisely the questions on which interpretation of form and pattern depend, whether examined from a physiological or a genetic point of view.

The seriation of stages of successive forming barbs constitutes a primary factor in the development of pattern by virtue of the changing level of reaction centers from barb to barb. The individual barb with its various associated parts is the element of form and pattern of the feather. Its rate of growth at different times in its formation is a second of the principal determining factors of form and pattern of the feather.

B. DESCRIPTIVE

The study deals exclusively with the adult feather. The method is the study of regeneration of plucked feathers; material of any age, in any desired abundance, may thus be secured at any time. We have made a special point of obtaining all possible information from entire mounts of regenerating feathers prepared in various ways. These are indispensable for a general view of the mechanics of feather formation. Serial sections of various stages, both transverse and longitudinal, have also been studied for various critical and finer points. Finally, the experimental results also offer critical evidence concerning developmental phenomena.

1. THE FEATHER PAPILLA

Regeneration, as has long been known, takes place from a papilla, attached to the inferior umbilicus of the feather at the bottom of the deep follicle. When the feather is plucked, the tip of the papilla is frequently torn off and there is slight bleeding into the follicle. The papilla consists of a dermal pulp covered with a layer of epidermal cells reflected from the bottom of the follicle (Plate I, Fig. 1); this

papilla measured 0.3 mm. from base to apex and 0.4 mm. in width at base immediately after plucking, and a papilla of a saddle feather 0.32 mm. in each of these dimensions. These are presumably about average dimensions. The epidermis of the papilla is from one to two cell-layers thick, and shows no sign of ridge formation; it is entirely lacking on the tip of the papilla immediately after plucking the feather. The pulp is rich in cells, soft and vascular, and exhibits no special regional differentiation. The feather papilla exhibits no further morphological organization at the time of plucking. However, its relation to the follicle enables one to distinguish its axis and the future dorsal and ventral sides (Plate I, Fig. 1).

It is certain that the feather papilla has such a simple structure for a large proportion of the time that intervenes between successive molts; but variations in germs of the same age after plucking suggest that further progress in organization may precede normal molting.

It should be clearly understood that we are speaking here of the succession of adult feathers whether as a result of plucking or normal molting, both of which are followed by regeneration. As Lynds Jones (1907) has so clearly shown, "the first down and its succeeding definitive feather are produced by one continuous growth," so that there is no cessation of activity of the feather papilla between their successive appearance. But the activity of the papilla of the definitive feather is periodic with a long resting interval between molts. No one has examined the papilla throughout this resting period, so that it has not been known whether its activity is resumed prior to actual molting, and in a sense as preparation for this, or whether the shedding of the feather is the actual stimulus to renewed activity. Measurements of feather germs made 3–5 days after plucking (see Table I) show, however, such a wide range of variation, and their corresponding degrees of differentiation may be so far apart, that we have no doubt that different stages must have existed prior to plucking. Occasionally a feather germ is found 5 days after plucking as far advanced as the great majority on the ninth day. We think it reasonable, therefore, to conclude that such exceptionally advanced germs belonged to feathers near the time of molting. This introduces an element of variability into all measurements timed from plucking,

which is, however, not either considerable or frequent enough to conceal the main features of growth-rates of different feathers.

2. THE TWO PERIODS IN THE DEVELOPMENT OF THE FEATHER

Immediately after plucking, the epidermis of the feather papilla begins to proliferate, and soon covers the exposed tip of the pulp. The papilla elongates, and the first barbs become visible as epidermal ridges longitudinally arranged on the surface of the pulp. The papilla has become the "feather germ." It comprises both epidermal and dermal components, and the term will always be used to include both. The superficial epidermal cells cornify to form the feather sheath which is finally sloughed off; the deeper epidermal cells between the sheath and pulp are concerned in the formation of the feather itself. Some features in the histogenesis of the sheath and of the barbs and barbules are indicated in Plate I, Figure 5, and Plate II, Figures 6–10; but these subjects have been so fully described by Davies and by Strong that we forego any description.

The entire feather germ and its sheath grow rapidly in length and soon project from the mouth of the follicle as the so-called "pin-feathers." These may attain a considerable length as such; but long before the entire feather is formed, the tips of the first barbs break through the apex of the sheath.

The data concerning the growth in length of the feather given in Table I and visualized in text Figure 2 show clearly that there are two sharply marked periods in the regeneration of the feather, the first characterized by a much slower rate of growth in length of the feather germ as a whole than the second. In the case of the breast feather, the transition comes on the ninth day after plucking; in the case of the saddle feather, after, or on, the tenth day. These two periods correspond to critical events in the morphogenesis of the feather, as will be shown below; the turning-point corresponds, in fact, in each case to the beginning of formation of the shaft by concrescence of the right and left halves of the collar. It is an interesting and significant fact that the growth-rates established after this time correspond precisely to the growth-rates determined by Juhn, Faulkner, and Gustavson (1931) after the tip of the feather germs appear beyond the mouth of the follicles on the surface of

the skin; these rates remain quite constant until the entire vane of the feather is formed, as the data and graphs of these authors show.

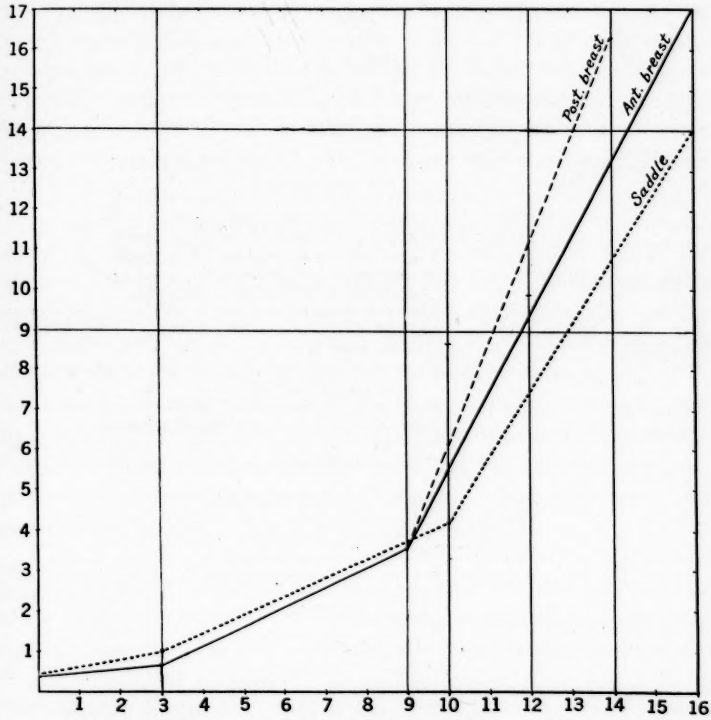


FIG. 2.—Brown Leghorn fowl. Rates of growth of the long axis of feathers. Abscissas, days; ordinates, millimeters. Constructed from data in Table I. In the case of the breast feathers the figures for anterior breast and posterior breast given *seriatim* in the table are plotted separately. The curves bring out the change in rate of axial growth between the first period of development before formation of the central rhachis and the second period of development after this event. The points on the tenth day for posterior breast and on the twelfth day for anterior breast are omitted in the curves. Similarly in the case of the saddle feathers the points on the twelfth and fourteenth days are omitted.

These facts make it necessary to divide the development of the feather into two distinct parts. The first period is concerned with the primordial organization of the feather germ, during which the

elements of the tip of the feather are laid down while the shaft is not yet formed; during the second period the shaft begins to grow and the remainder of the vane is formed. It is possible to say precisely how much of the tip of the feather is formed during the first

TABLE I

MEASUREMENTS TO 16 DAYS AFTER PLUCKING

Days after Plucking	Length in Mm.	Remarks
I. Breast:		
0.....	0.32	Papilla immediately after plucking
3.....	0.64	Average of 11; range from 0.5 to 1 mm.
5.....	2.16	Average of 10; range from 0.6 to 4.75 mm.
7.....	3.2	Average of 14; range from 1.7 to 4 mm.
9.....	3.5	Average of 20; range from 3 to 4 mm. Rhachis begins to form
10.....	8.7	Average 8; range from 7.5 to 9 mm.—to tip of dorsal barbs, *post. br.*
12.....	9.9	Average of 15; range from 8.5 to 12 mm.—to tip of dorsal barbs, *ant. br.*
14.....	16.3	Average of 12; range from 11.5 to 19 mm.—to tip of dorsal barbs *post. br.*, sheath ruptures
16.....	17.0	Average of 12; range from 14.5 to 18 mm. to tip of dorsal barbs, *ant. br.*
II. Saddle:		
0.....	0.32	Papilla immediately after plucking
3.....	0.97	Average of 7; range from 0.6 to 2 mm.
5.....	1.4	Average of 7; range from 0.6 to 2.5 mm.
7.....	2.5	Average of 13; range from 1.5 to 4 mm.
9.....	3.9	Average of 6; range from 3 to 4.5 mm.
10.....	4.1	Average of 5; range from 3 to 5 mm.—to tip of dorsal barbs, rhachis begins to form
12.....	6.85	Average of 12; range from 4.5 to 8 mm.—to tip of dorsal barbs,
14.....	10.2	Average of 10; range from 6 to 12 mm.—to tip of dorsal barbs
16.....	14.1	Average of 14; range from 11 to 16 mm.—to tip of dorsal barbs, sheath not yet ruptured

period, as will appear later; and this may be of significance with respect to the physiology of formation of tip patterns, as in the spangled breeds of fowl for instance.

3. THE FIRST PERIOD

This period lasts about 9 days in the breast feather, 10 days in the saddle in agreement with the generally lower rate of growth in the latter (Juhn and Gustavson). As certain features are peculiar to

each type of feather, we shall consider them separately, beginning with the breast feather.

a) *The breast feather.*—It will be simplest to consider the condition existing on the eighth and ninth days first, and then to take up the development of this condition as needed. Plate I, Figures 2 and 3, are photographs of an entire feather germ 8 days after plucking, at a magnification of 11 diameters to show the entire germ and at 30 diameters to show some of the details of the pigmented ridges (barbs). The photographs are taken from the dorsal surface and show at *X* the earliest stage in the formation of the rhachis. It will be noted that all ridges appear to end separately at both ends. However, the unpigmented bases of the ridges continue into the collar, which extends to the umbilicus. The ridges shown are all of approximately the same length, but on the opposite surface of the germ they taper off to successively shorter ridges always attached to the collar. This view cannot be successfully photographed *in toto* because the ventralmost ridges, which are also the youngest, are entirely unpigmented. The dorsalmost ridges have attained their full plasmatic growth (see p. 150) at this time, but the series extending ventrally from them have regularly increasing increments still to make during their migration to the mid-dorsal line (see p. 146).

Plate II, Figure 8, is a transverse section of a practically identical germ 9 days after plucking, taken as close as possible to the bases of the ridges, thus below or at the margin of the pigment shown in Plate I, Figure 2. The mid-ventral line is recognized by the lowness of ridges, and the mid-dorsal line by two fused ridges which mark the beginning of the shaft. This fusion extends only 0.25 mm. forward, and these ridges then become free like the others. The fusion involves only the bases of the two mid-dorsal ridges and one other at a slightly higher level. The pulp is somewhat shrunken in the preparation; but in places the extension of the pulp between the ridges can be seen; prolongations of pulp cells extend in fact to the bases of the ridges as seen in cross-section. The number of ridges is 104, of which 48 are on the right side of the dorsoventral plane and 56 on the left, thus a pronounced asymmetry, to be referred to later.

Plate II, Figure 9, is a section 0.61 mm. in front of Plate II,

Figure 8, from the same series. The 10 shortest ridges have already disappeared, and only 94 are left. One cannot distinguish the mid-dorsal line, as the section is in front of the rudimentary rhachis. The detail of a few of the ridges is shown in Plate II, Figure 10. The pigment cells (*c.*) feed pigment into the barbule cells (*b.*); this process has been excellently described by R. M. Strong (1902), and detailed descriptions will be omitted. It should, however, be noted that the individual barbule cells shown in such a section belong to successive barbules (cf. Plate II, Fig. 6), as the section cuts across the obliquely arranged chains of cells of the barbules themselves.

Plate I, Figure 4, is a sagittal section of an 8-day germ from the breast; and Plate I, Figure 5, and Plate II, Figure 6, give the details of the base and of one of the ridges at magnification noted in the captions (Plate I, Figure 3, is to be used for orientation on the points noted in the caption). The dense growing zone at the base of the pulp should be noted and the gradual loosening in the texture of the pulp apicalward, as well as its vascular character. Plate I, Figure 5, showing the relations at the base, requires no description. Plate II, Figure 6, is a paratangential section along a ridge, in the position marked in Plate I, Figure 4, which shows nicely the pigment cells and the forming barbules, each composed of a chain of single cells. The distances between successive forming barbules will be a point for discussion later.

The stage of the breast feather just described is at the end of the first period just before rapid growth sets in. It remains now to discuss the development and the morphological significance of this stage.

The ridges formed in the first stage represent the barbs of the apical part of the definitive feather; numbered from the mid-dorsal line, they correspond serially to the barbs of the definitive feather numbered from the most apical barb backward. It is thus somewhat surprising to find at this stage that approximately 50 ridges are already formed on each side (leaving the question of asymmetry aside for the present), representing approximately the anterior half of the vane of the feather.

But this first period itself, so far as the formation of the barb primordia is concerned, must be divided into two stages. The dis-

tinction which we shall make is between the first ones that arise practically simultaneously and those that arise ventrally to these in serial order. It is difficult to be certain about this situation, and to say just how many barbs belong to the first and how many to the second class; but the logic of the situation creates the distinction, for we start out at first with an entirely unoccupied field all around the circumference of the base of the feather germ, and the first barbs appear to form *in situ* in about the dorsal two-thirds of this field, not next the mid-ventral line as the later ones do. Our material covering these stages is not exhaustive, but we have never yet seen a stage that was not either entirely devoid of ridges or, on the other hand, provided with a considerable number. The question may well deserve further examination in connection with the problem of apical patterns. For the present we would estimate that about 25 barbs form practically simultaneously on each side and that the remainder arise *seriatim* in the ventral field.

The manner of origin and growth of barbs seems thereafter to be at all times the same, and we shall therefore leave the discussion of such questions as the movements of barbs toward the rhachis, their apico-basal order of formation, and gradients of growth, until our consideration of the second period of development; but we should deal here with the problem of the growth centers, or organization centers, of the barbs. In order to do this, we must go back and consider the primary organization of the feather germ with special reference to the basal portion of its epidermis which we have called the "collar."

It is readily seen that with the formation of the first barbs the feather germ is divided into three epidermal zones (Plate I, Fig. 2): a middle one occupied by the barb primordia; an apical zone without barbs, where the pulp is constantly dying off; and a zone basal to the barbs, which includes the collar, where, similarly, no barbs exist. This zone is the formative zone of both barbs and shaft. It is aptly called the "collar" because it completely surrounds the pulp and is of relatively small height at all stages. At its posterior margin the cells of the collar are continuous with the epidermis of the feather follicle (Plate I, Fig. 5); at its anterior margin the bases of the barbs are attached.

The collar in a broad sense consists of many layers of small epidermal cells (Plate II, Fig. 7), those next the pulp corresponding, of course, to the Malpighian layer; at the stages illustrated the external cells are already keratinized to form the cells of the feather sheath (Plate II). The cells corresponding to the Malpighian layer constitute the collar in the strict sense, and the term will be used in this sense hereafter. The collar is the exclusive source of both barbs and shaft. This of course implies growth centers for both. Those of the barbs are serially arranged along the anterior margin of the collar (cf. text Fig. 1). New ones arise only ventrally, never between adjacent centers. The undifferentiated cells just posterior to the barb centers and continuous with them are the primordium of the shaft. Its growth starts in the mid-ventral line and is dorsally directed on each side. The result of its activity is a constant dorsally directed flow of cells that carry the barb centers along with it. The original continuity of barb centers and shaft primordium is permanently preserved. In the next section the analysis of this situation, and the consequences that result from it, will be considered more fully.

If one goes back to the feather papilla just after plucking (Plate I, Fig. 1), it will be seen that the epidermis is a very thin layer with no indication of collar or other differentiation. We have not followed the intervening stages, but it is obvious that great activity of cell division and growth must begin in the epidermis of the papilla very soon after plucking.

It must be emphasized that the collar is the center of all formative activity of the feather. We find, correspondingly, that cell division is very active in it throughout development. The distribution of such divisions with reference to the growth centers that we have distinguished is not, however, very obvious, save in the extreme bases of the barb ridges, where their very rapid elevation into the pulp is prepared. Here one sees numerous division figures, indicating a very rapid cell multiplication. The cells, however, appear to aggregate to form these successive centers without the appearance of an unusual rate of cell division, with reference to its occurrence in the collar as a whole. The zone of cell division appears to have a very limited extension anteriorly in each barb, and ceases entirely at the level where pigment appears in the pigment cells. This situa-

tion is significant in connection with the distinction made in the next section between growth by cell multiplication and growth by differentiation in the barbs. So far as the growth of the shaft part of the collar is concerned, there is no clear evidence of a special concentration of division figures in the extreme ventral part, but division figures seem to be scattered fairly uniformly throughout its entire extent from ventral to dorsal up to the point of concrescence (see section 4), just above which cell division ceases and differentiation begins. There must, nevertheless, be a point at the mid-ventral line where the plasmatic growth is started in two opposite directions. An experimental determination of this interesting question is theoretically possible.

b) *The saddle feather.*—The specific features that differentiate the saddle feather from the breast feather of the cock, capon, or poulard appear in the feather germ at a very early age. Apart from a slower rate of growth as a whole, these are, on the morphological side, the different form, the entire absence of barbules from the most distal barbs and their restricted distribution over the remainder of the vane in a long axial acuminate zone gradually broadening toward the base, the resulting "lacing" of the vane, and the beautiful golden coloration of the lacy part of the feather. The general principles of development are, of course, the same as in the breast feather. However, in the lacy areas, which include the total length of the first twelve barbs, at least, neither barbule cells nor branched chromatophores differentiate; but the fact that it is possible to induce their development experimentally (see p. 170) is good evidence that such cells of both kinds are present in an undifferentiated condition, and for this conclusion there is also cytological evidence. The same conditions are found in the barbule-free sections of the succeeding barbs. Wherever barbules are formed, branched chromatophores also occur, feeding pigment into the barbule cells as in the case of the breast feathers (Plate II, Figs. 6 and 10). It is difficult to determine whether the pigment found in the naked barbs of the male-type saddle feather is derived from the same chromatophores, but in a relatively undeveloped condition, that supply the pigment of the barbules. There is the possibility that pigment forms *in situ* in the cells of the barb itself.

In addition to the foregoing differences the greater apparent width of the ventral zone of the germ of the saddle feather in comparison with the breast feather is very striking (Plate III, Figs. 16 and 19). This is due in large part to the lowness of the ridges, which is associated with the absence of barbules.

4. THE SECOND PERIOD

a) *The shaft.*—After the formation of ridges arising during the first period, the development of succeeding ridges or barbs is associated with the formation of the shaft. The part of the collar in which the bases of the primary ridges end is the primordium of the shaft, which thus has the form of a ring. The shaft itself forms by concrescence of the halves of this ring in the mid-dorsal line, and the growth energy is furnished by the growth and multiplication of its cells directed inversely from the mid-ventral part of the ring. This growth carries the bases of the barbs and their individual growth centers continuously dorsalward on each side; the rate of this growth, and consequently of the movement of the barb centers, is so regulated that by the time the base of a given barb reaches the mid-dorsal line the barb is completed. There is thus no time in its development when the barb is not attached to the primordium of the shaft.

The identification of the two halves of the collar as shaft primordium is rendered certain by the attachment of the developing barbs here, and by the arrangement of parts at the place of concrescence. This is shown in Plate III, Figure 12, where the gradual transition of barbs from those attached to the collar to those attached to rhachis is clearly illustrated. The arrangement of barbs as shown is a space arrangement, but it also indicates a time arrangement, as will be discussed more particularly in section 4, *c*, *i*.

It was stated by Davies (1889) that the apex of the shaft is formed by fusion of ridges in the center of the dorsal side; in this interpretation he was followed by Strong (1902), who says that "the shaft or rhachis . . . . represents two or more combined ridges." This is, however, incorrect. The dorsalmost ridges of the original series form the two most apical barbs projecting beyond the apex of the shaft. It is clear from Plate I, Figure 3, that during the first

period the two dorsalmost barbs are laid down and become pigmented and keratinized while separate from one another. After that there can be no fusion except at their undifferentiated bases; it is at their bases, as a matter of fact, that the shaft proper begins by a fusion of the two half-rings of the collar, to which the bases of the first barbs, as well as those of all succeeding barbs, have a primary attachment.

The special morphology and histogenesis of the shaft fall outside the scope of this paper. For information on this subject consult Davies (1889).

The spacing of the barbs along the rhachis of the definitive breast feather is of the order of ten times the spacing while they are still attached to the collar.[1] This means that 90 per cent of the growth of the rhachis in length is accomplished after concrescence. As multiplication of cells ceases at the point of concrescence, or slightly above, this growth is by processes of differentiation of already formed cells. The elongation of superficial cells and the extraordinary "ballooning" of the pith cells furnish sufficient explanation of this remarkable fact. The feather furnishes a splendid opportunity for estimating amounts of total growth in a specific process of organ formation due to processes of cell multiplication and cell differentiation respectively.

Juhn, Faulkner, and Gustavson's measurements of rates of growth (1931), which are derived from measurements of the length of the axes of feathers at different times, are thus composed of 90 per cent of growth by differentiation of the shaft cells and 10 per cent of growth and multiplication of cells of the shaft primordium in the collar. Moreover, the reactions of the feathers to the female hormone, which they find proportional to rates of growth so determined, are exhibited in the vane almost entirely, and are thus functions, not of rate of growth of the shaft, but of the barbs, which are not involved in their measurements. Nevertheless, there can be no doubt from their findings that the threshold of reaction to the female hormone can be expressed as a function of the rate of growth

[1] The distance between successive barbs of a particular definitive breast feather was found to average 0.42 mm., somewhat more toward the apex of the shaft and somewhat less toward the base. In a 10-day feather germ of corresponding location the distance between successive barbs still attached to the collar was found to average 0.044 mm., somewhat more toward the dorsal side and somewhat less toward the ventral side.

of the axis of the feather. There must therefore be a close correlation between the growth rates of the rhachis center and of the barb centers. Something sets the pace, so to speak, for the feather as a whole. This subject is discussed again under growth of barbs (p. 148).

We have given the name "collar" to the ring of formative cells, surrounding the base of pulp, from which both rhachis and barbs are derived. Along its anterior margin are the formative centers of the barbs; next to these basally is the continuous formative material for the rhachis, consisting of two half-rings united at the mid-ventral line. Growth movements directed dorsally on each side maintain the constant streaming, which carries the bases of the barbs along with it, and results in concrescence of the two halves in the mid-dorsal line to form the rhachis with its attached barbs. The primitive embryonic form of the feather is thus that of a ring with attached barbs; and the axis of the feather arises by outgrowth, due to concrescence, from the mid-dorsal surface.

Davies' conclusions as to the phylogeny of the definitive feather, which he based on a comparison of the development of the earliest down and definitive feather, were very similar as regards the shaft. The most primitive form of the feather he considered to have been a corneous ring (*Spule*) bearing rays symmetrically along all of its upper surface. The basal opening of the *Spule* was the lower umbilicus, the apical opening to which the rays were attached the upper umbilicus. The following passage (1889, p. 633) summarizes his view as to the evolution of the definitive feather with its shaft:

> The great distinction between the primitive down and the definitive feather, apart from the aftershaft, is as follows: while in the primitive down the upper border of the *Spule*, to which the rays are fastened, forms a circle, in the definitive feather, in consequence of enormous prolongation of one side, it forms a long drawn-out obliquely placed ellipse, and by the great thickening of this prolongation the upper umbilicus is almost completely closed. In this manner there arises in the definitive feather a structure, the shaft, which in reality is only a part of the *Spule*, although it makes the impression of an entirely new structure.

Farther on he says, "But all parts which bear rays are only differentiated or specially developed parts of the original *Spule*."

b) *The pulp.*—It will be seen from Plate I, Figure 3, that during the first period the pulp is much longer than the ridges formed upon

its surface. At all stages the pulp exceeds in length the barbs in process of formation; hence the pulp is continuously growing in length, as the series of barbs become progressively longer to correspond to the taper of the feather, until it attains a length surpassing that of the longest barbs. This results in a relatively enormous extension of vascular tissue from the bottom of the follicle which is protected by the feather sheath and the layer of barbs within. The growth in length of the pulp corresponds at all stages to the lengths of the barbs which determine the taper of the formed feather; there is thus a strict correspondence between the lengths attained by the barbs to insure the taper of the feather and the growth of the pulp. Prospectively considered, we may say that the required length of barbs determines the elongation of the pulp. There is a beautiful synchronization of these processes to produce the several feather types.

In making the spread preparations shown in Plate III, Figures 11–19, the feather germ is split on one surface from base to apex, and the pulp removed. The length of the pulp can then be accurately ascertained. In the case of the breast feather at 16 days, the length is found to exceed 17 mm., which corresponds to the length of the longest barbs. The barb depends for its nutrition upon an intimate relation to the vascular pulp, and this relation is not suspended at any time during the growth of the barb. As soon as the growth is completed, the barb frees itself from the pulp.

As all barbs take their origin from the "collar," that is, at the base of the pulp, each grows from the base to near the apex of the pulp before its growth is completed. Either the series of growing barbs shifts continually on the surface of the pulp or the pulp itself has growth processes corresponding to the growth processes of the series of barbs. Now the relation of the pulp to the ridges is a very intimate one, as shown by the way it dips in between them, and molds every inequality (cf. Plate II, Figs. 8–10). When the wall of the feather germ is split longitudinally and spread out, and the pulp turned back from its apex, parts of ridges always stick to the pulp and come away with it, so intimate is the connection. Physiological considerations also would seem to demand continuity of relationship. It is therefore very significant that, as Davies points out, the densest

portion of the pulp is in the region of the collar and that there is a loosening of texture of the pulp in an apical direction (cf. Plate I, Fig. 4). The growing part of the pulp, as of the epidermal portions of the feather germ, is basal. We must thus think of the pulp as constantly growing at its base, and constantly dying and being resorbed at its apex. It may even possess growth-rates in its various parts corresponding to the curve of growth-rates of the barbs. The correlation of growth processes in the formation of the feather certainly extends in a very exact way to the relations between the epidermal and the dermal components.

c) The barbs and barbules.—

i) Seriation of barbs: From what has already been said, it seems hardly necessary to emphasize that the time order of origin of barbs is strictly serial from the apex to the base of the feather, so that each more apical barb is a little older than the next basal. This seems to have been understood for a long time, and is clearly implied in the statements both of Davies (1889) and Strong (1902). The only possible qualification of this statement relates to the first barbs formed in the first period of development (see p. 137). The first of these, as we have seen, appear to arise simultaneously, although we are not prepared to state in an unqualified manner that there may not be a slight time seriation in these also.

In the second period or development, now under consideration, all new barbs arise next to the mid-ventral line, from centers of growth situated in the anterior margin of the collar as in the case of the barbs formed during the first period. Room is made for succeeding barbs by the dorsally directed growth movement in the collar (primordium of the shaft), which carries the bases of the barbs along in a constant stream on each side until they join the rhachis. Plate III, Figures 13 and 16, are photographs of spread-out preparations of germs of breast feathers of 10 and 12 days after plucking, respectively, split along the dorsal side. The critical ventral basal region is shown enlarged in Plate III, Figures 14 and 17. The breast feathers of the cock or capon are especially favorable for such preparations as the barb ridges become heavily loaded with black pigment so quickly; nevertheless, the youngest ridges, which are yet unpigmented, do

not appear clearly in the photographs. Sections (Plate II, Figures 8 and 9) show that those of the right and left sides are almost in contact.

We know from the work of Juhn, Faulkner, and Gustavson (1931) that the time required to form the vane of a breast feather is approximately 20 days from the time of plucking. On the average, there are about 100 barbs on each side in the vane of an anterior breast feather. If we eliminate the first 9 days as the period required to form the barbs, about 50 in number, in the first period of development, 11 days are required to form the 50 remaining barbs of the vane which arise during the second period; between 4 and 5 barbs therefore arise each day during the second period on each side. As there are between 40 and 50 forming barbs from youngest to oldest on each side, it follows that each barb requires about 10 days to complete its formation.

A second method of estimating the time required for growth of a barb is given by interrupted injections of the female hormone. In the case of the breast feathers, interruption of 1 day in 7 gives male bars in the breast feathers of the capon on a female background (Juhn and Gustavson, 1930). The anterior edges of two successive male bars are therefore 7 days' growth apart. If a single barb near the middle of the vane is followed in its oblique course across the vane, beginning at the margin where the anterior edge of the first male bar intersects it, it is found to cross over into the second male bar and to join the rhachis at its posterior margin. Such a barb therefore exhibits 7 days' growth from its male tip through the female section to the anterior end of its male base. To obtain the total time occupied in its growth, we have to add the time occupied by the male bar. This is more than 1 day's growth, although only 1 day was omitted in the injections, because, as has been shown by Juhn, Faulkner, and Gustavson (1931), the breast feather has a very high hormone threshold, and it requires at least 2 days' injection of the female hormone to restore the hormone level at which the female reaction begins again with the dosages used in this particular experiment. And, if one compares the width of the male bar to the sum of the widths of the male bar and female bar of a week's growth (Juhn and Gustavson, 1930), it is found that the male bar is more than one-third

of the total, that is more than 2 days' growth. It is necessary, therefore, to add approximately 3 days, giving a total of 10 days occupied in the growth of a single barb. This estimate by an entirely different method thus agrees with the estimate reached by the other method.[1]

These time relations are very significant for the physiological results. It should be mentioned that the shorter barbs at or near the tip of the feather do not extend through two male bars. Where the feather is male-tipped, the first 10 barbs (or so) do not even extend through the male tip; hence the time of their formation is relatively brief corresponding to their length, which ranges from 1.5 mm. to 5 mm. only.

ii) The growth and migration of the barbs: This subject can best be understood if we follow the history of a single barb from the time of its origin next to the mid-ventral line until it joins the rhachis, when its growth is completed. It arises as a very short unpigmented ridge representing the most apical portion of the definitive barb. It possesses a growth center at its base in the collar, which is constantly adding to its base, so that the order of origin of its parts is strictly from apex to base, and this naturally determines the gradient of differentiation. The processes of cell multiplication in the barb center and of differentiation of cells above the center determine its growth in length on the surface of the pulp. As it grows in length, its attached end undergoes a separate movement constantly dorsalward with the flow of the rhachis primordium. Inasmuch as its apical end is not carried along, but preserves an approximately mid-ventral position, when the base reaches the rhachis its length describes a half-spiral around the cylindrical pulp. This arrangement has often been observed; its beginning will be noted clearly in the ventralmost pigmented barbs of Figure 17, Plate III.

Thus two kinds of movement determine the position of the barb at its various stages with reference to the pulp: (1) the movement of

[1] Two specific measurements are given here for illustration: barb No. 57 on the right in a particular feather is 1.7 cm. long. It takes in two black bars, at apex and base respectively, with the intervening buff (♀) section. In another feather, barb No. 69 on the right is 2 cm. long; it similarly occupies two black bars at apex and base respectively with the intervening space.

its base with the collar from the mid-ventral to a mid-dorsal position; (2) the simultaneous growth of the barb in length. These movements are due to the simultaneous activity of two kinds of growth centers—that of the rhachis primordium and that of the series of barb centers at the base of each growing barb in the anterior margin of the collar.

The apex of the barb is the first part formed, and growth in length takes place by constant multiplication of cells of the growing center of the barb situated at its base in the collar. Thus the seriation of stages for the barb, as for the rhachis, is in an apico-basal order. Thus the oldest part of the rhachis is at the tip of the feather, and the oldest part of the barbs at the feather margin. Just as the order of age of the barbs is from apex to base along the rhachis, so the order of age of the barbules is from margin to shaft along the diagonally arranged barbs. This is more fully confirmed by the experimental results given in Part III. Thus we have two time gradients within the feather—the one along the rhachis, the other along the barbs. These constitute the fundamental bases of reference for all developmental events.

It of course also follows that the ventral barbs at any given stage of the germ represent the marginal portion of the completed vane to an amount proportional to their degree of incompleteness. Events in the ventral region of the feather germ are thus to be referred to the margin of the definitive vane.

As new barbs are constantly forming, at the rate of 4 to 5 per day in the case of the breast feathers, the consequence is that the collar is thickly set at any given time with a veritable picket fence of barbs increasing in height on each side from the mid-ventral line all the way to the rhachis. As the part of each barb that is alone susceptible to morphogenetic reactions is situated in the immediate neighborhood of its growth center, all determinations being fixed above this, it will be immediately evident, first, that all determinations occur while the barb is attached to the collar and cease at the time it joins the rhachis; and, second, that at any given time the reactive region of any barb of the existing series is a function of its position in the collar, being progressively more apical in position in the dorso-ventral order (see diagram, text Figure 1). This principle furnishes the bases of all physiologically determined feather patterns.

The principle may be expressed thus: Any given morphogenetic reaction takes place in each barb concerned at equal physiological age. In a series of barbs affected simultaneously, the points of equal physiological age will come to be successively farther from the rhachis in the definitive feather in descending order, because this is the order of age of barbs and because differentiation in the barbs is apico-basal in order. On account of the oblique arrangement of barbs to the rhachis of the definitive feather, points of equal physiological age in the germ lie in a line approximately at right angles to the rhachis of the definitive feather.

iii) Growth by cell multiplication and by cell differentiation in barbs: We determined the relative amounts of growth in length of the shaft due to cell multiplication and to cell differentiation respectively by comparing the spacing of barbs in the collar and in the definitive feather. A similar determination can be made for the barbs by the spacing of barbules at the time of their formation and in the definitive barb, for there is no growth by cell multiplication after the barbules are once layed down. It can readily be determined by the study of such a preparation as is shown in Plate II, Figure 6, that the barbules at the time of their first formation are only about 0.006 mm. apart. Although there is some variation in the spacing of barbules along a given barb of the definitive feather, it is really very slight. The average distance apart in the breast feather is 0.05 mm. After cell division had ceased, therefore, the barb itself had grown 9.5 times its original length. This is in very close agreement with the figures obtained for the shaft.

iv) Gradients of growth-rates in the individual barbs: The experiments described in Part III require for their interpretation the assumption of a gradient of growth rates in the formation of a barb, relatively high at the apex and diminishing toward the base. After much effort a definite independent proof of this assumption has been found and will be described here; but it seems worth while to mention first quite briefly methods that failed, because they bring out certain significant principles.

First, it was realized that, by measuring the length of every fourth barb-forming ridge of a feather germ of appropriate age, daily increments of growth could be obtained on the assumption that all barbs behaved alike; and there was no objection to this assumption,

provided that the middle region of the vane where barbs are of substantially equal length were selected. There are considerable technical difficulties in obtaining these measurements, but it is possible to obtain them. They show a rapidly decreasing daily percentage of growth increment from youngest to oldest forming barbs which is apparently the principle looked for. But when it was realized that about 90 per cent of the growth is made up of growth by differentiation of already determined cells, and that the rate of growth we are interested in is the growth by cell multiplication in the growth centers, it became impossible to place exclusive reliance on these figures.

The second method of attempting to estimate rates of growth at successive levels along the axis of the barbs is equally uncertain. This method depends on the transverse male bars produced in breast feathers by interrupted injections of the female hormone. Such bars cut across the barbs obliquely from rhachis to margin. If the time during which the reaction lasts were the same for all levels of the barbs, then, if the rate in the zone of growth suffered a decrement from apex to base of barb, such a bar should widen toward the margin from the rhachis, for it passes successively across more apical sections of the barbs from rhachis to margin. As a matter of fact, many bars do widen in this way; and it may be that this phenomenon is to be interpreted as due to more rapid growth in the apical zone. This method, if the foregoing assumption were trustworthy, would be particularly demonstrative, because, as will be shown more particularly in Part III, the reaction is determined finally in the zone of growth. However, the female reaction requires a higher threshold of hormone in the apical region of the barb (see Part III). The factors of reaction rate and hormone threshold work in opposite directions, and this renders uncertain any conclusion concerning rates of growth at different levels of the barb that are based on the form of the bar.

After a concentration of hormone adequate to satisfy the apical thresholds is once attained, the reaction rate is much greater for the higher thresholds ventrally than for the lower thresholds dorsally. The reaction lasts longer dorsally, owing to the curve of concentration depending on processes of absorption and excretion, and pro-

ceeds more rapidly ventrally; and this may result in identical lengths being affected dorsally and ventrally.

The third method of measuring growth rate does not appear to be open to any of the foregoing objections. It depends on the fact that the female hormone dosage may be so adjusted that it affects the reactions of all the barbs simultaneously (see p. 166). If, then, the feather germ be plucked 48 hours after the injection, the reaction is found recorded in the entire reaction zone, a narrow band extending across the entire base of the feather germ widening ventrally (text Fig. 1). As successive barbs correspond to successive apico-basal levels of a single barb, the reaction is recorded from a basal position in the dorsalmost through all intermediate positions to an apical position in the ventralmost forming barbs. After establishing this band, the injections cease and the reaction reverts, leaving a base of reference for measuring subsequent growth.

In preparation for the experiment areas of feathers are plucked about 2 weeks before the injection; regenerating feathers of equal age are thus present. Samples are plucked day by day after injection and prepared. The base-line mark, which is permanent, now records day by day the amount of growth basal to it for each barb. If rates of growth were equal for all levels, such a base line should continue to be parallel to the collar. If the rates of growth were not equal for all levels, the base line would cease to be parallel to the collar. It is obvious in general that, where growth rates are greater, levels of differentiation (or reaction) must be at greater distances from the growth centers, i.e., from the anterior end of the collar. Thus, if the ventral growth centers are more active, the band formed in the manner just described must be bent away from the collar at its ventral end. This is, in fact, the case.

When this is once recognized, it becomes obvious that the explanation of the ventral triangle shown in Plate III, Figures 13, 14, 16, and 17, which is marked by the normal deposit of pigment in the breast feather is the same. The advantage of basal band established in the manner just described over the merely normal condition consists in being able to follow it in subsequent growth processes. The band may in fact be interpreted as a curve measuring growth-rates at successive levels of a barb.

It is necessary to examine this subject in detail; and we shall begin with the breast feather, using the female hormone as indicator, and then proceed to the saddle feather, using thyroxin as indicator.

(*a*) The breast feather—base lines established by injections of female hormone: Prior to injections the barbs are black; the female hormone changes the color abruptly to salmon. If the dose of hormone injected is properly calculated, this change occurs nearly simultaneously in all forming barbs. The first visible reaction is sharply restricted to a narrow zone a short distance above the barb centers, i.e., the junction of barbs and collar; as already indicated, the zone bends up ventrally (Plate IV, Figs. 20 and 21). So long as the concentration of female hormone in the blood remains high, the salmon band increases in width; when concentration falls sufficiently, the barbs revert to black (Plate IV, Figs. 24 and 25); thus the second base line is established, the first base line being the anterior margin of the female (salmon) bar. Rates of growth along the collar can be established by reference to either base line; but they must be considered independently, because the first is established by a sudden change (injection of a large amount of female hormone in a short period) and the second by a gradual change (excretion of the hormone). The two base lines can be established any distance apart by varying the number of daily injections.

Capons are used for these experiments; different birds vary greatly with reference to their sensitivity. In one experiment carried out identically on two birds, one (29–970) gave beautiful reactions in all feathers, the other (29–35) gave only fair reactions in a few feathers, and no reaction at all in others. It is therefore important to use birds the reactivity of which is known by previous experiments.

The actual growth centers are all on the same level with reference to the collar (text Fig. 1). The higher level of pigment deposition in the region of the ventral triangle of the normal feather is evidence of more rapid growth in the most ventral barbs of the feather germ, which are apical regions of definitive barbs.

As the base line in the experiments is established by a change in color of pigment deposited, it is obvious that even at the start it must bend up in the region of the ventral triangle. This form is maintained or accentuated during several days after establishment

of the base line, thus indicating that the rate of growth of apical regions of barbs remains greater than elsewhere.

Text Figures 3–6 are drawings from photographs in Plate IV, Figures 20–23; text Figures 7 and 8 are drawings from photographs in the same series not reproduced.

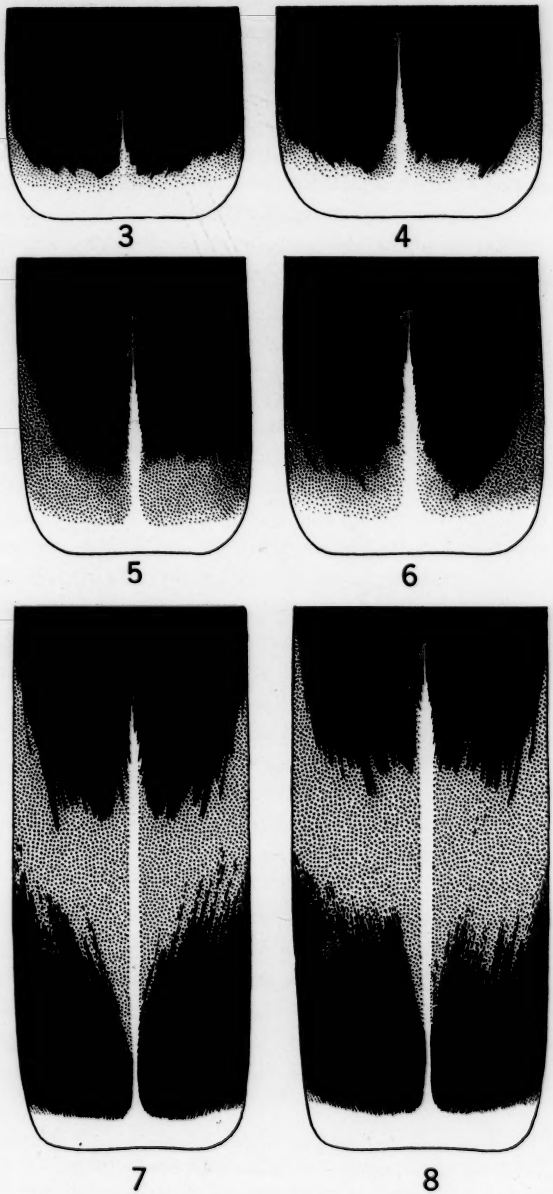
Text Figures 3–8 show the form of the first base line at 3, 4, and 7 days after the first of three daily injections; on the sixth day the second base line has appeared, and the full width of the female band, established by injections on three successive days, is formed.

The first base line measures the growth from the collar taken as a base in a known period of time. The form of the line thus represents a curve of growth rates. It is obvious that the fastest rate of growth, which is ventral, is at first several times the slowest rate, which is intermediate and dorsal. The curve is very steep at the ventral side and rapidly becomes almost horizontal all the way to the rhachis 96 hours after the first injection (Plate IV, Figs. 22 and 23, text Figs. 5 and 6). At its highest point the ordinate to the base line is about four times the ordinate in the almost horizontal part in this case.[1] If we transfer this result to the individual barb, we reach the conclusion that in each barb the apical growth-rate is at least four times that of intermediate and basal portions of the barb, and that there is a steep gradation from the most rapid to the average rate of growth. In terms of the width of the vane of the definitive feather the margin is derived from the most rapidly growing part of the germ, and the proportion of the most rapidly growing to the average rate is from one-quarter to one-third of the entire width of the vane. This checks very well with patterns formed in breast feathers by injection of female hormone in varying doses (see Part III).

At least four of these base-line experiments have been performed at different times. A considerable number of feather germs have been studied from each, and the results found in each case show the more rapid rate ventrally.

(*b*) The saddle feather—base lines established by injections of thyroxin: Juhn and Barnes (1931) have published a paper on the

[1] On account of the never ceasing movements due to growth in length, and migration of barbs dorsally, and also on account of unknown factors governing deposition of pigment—whether, for instance, a given chromatophore may form both kinds of pigments at different times, how long reactivity lasts in a chromatophore, etc.—this quantity must be accepted with reserve. It may be greater in reality.



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FIGS. 3–8.—Drawings from photographs (Plate IV; see for protocol), illustrating the development, after female hormone injections, of base lines establishing rates of growth in breast feather germs and the asymmetry of the female reaction (represented by stippling) on the two sides.

FIGS. 3 AND 4.—Right and left feather germs 3 days after the first of three daily injections.

FIGS. 5 AND 6.—Right and left feather germs 4 days later.

FIGS. 7 AND 8.—Right and left feather germs 7 days later.

feather germ as indicator of thyroxin in the blood. We have used this reaction for base-line determinations of growth-rates in accordance with the principles just discussed for the reaction of the breast feather to the female hormone. The authors just quoted did not discuss the nature of the thyroxin reaction. It involves two definite processes: first, the formation of black pigment, which is normally restricted to the base of the saddle feather; and second, the extension of barbule formation, which is normally confined to the bases of the barbs, toward, and even to, the tip of the barbs. These processes are more or less interdependent, but barbule extension proceeds at a lower threshold than the deposition of black pigment, thus producing interesting patterns more fully discussed in the next section. In the normal saddle feather chromatophores such as those illustrated for the breast feather in Figures 6 and 10, Plate II, are either entirely absent or quiescent (see p. 141). Injection of thyroxin in sufficient concentration, however, causes them to appear in a form strikingly like those of the normal breast feather, loaded with black pigment and provided with similar processes. They also deposit their pigment into the barbule cells which arise simultaneously just as in the normal breast feather.

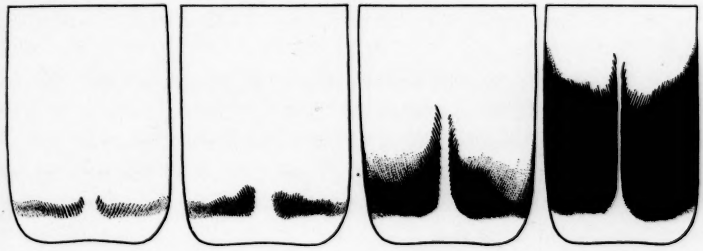
It is this process of deposition of melanin with hyperthyroidism that we use for establishing our base line. With a sufficient dose of thyroxin a black band appears practically simultaneously near the bases of the barbs, at all levels from the dorsal to near the ventral surfaces of the feather germ.[1] This is our base line (cf. Plate IV, Fig. 41, text Fig. 9). In a period of 4 days after the injection the base line describes a curve from ventral to dorsal surfaces, which at first descends sharply, and then more gradually, but always definitely descending (Plate VI, Fig. 44; text Fig. 12). This defines the gradient of growth-rates from apex to base of the individual barb, which therefore descends all the way to the rhachis; it does not strike a practically dead level at a considerable distance from the rhachis as it does in the case of the breast feather.

[1] It must be remembered that the growth of the saddle feather is considerably less rapid than that of the breast feather. It might be expected, therefore, that the ventral end of this line would not form so great an angle at first with the remainder of the base line as in the breast feather, and this is actually the case.

These differences in gradients of growth-rate are reflected in the patterns that can be induced in the two kinds of feathers, as will be explained in the next section.

5. ASYMMETRY OF THE FEATHER GERM AND THE DEFINITIVE FEATHER

When we come to consider the feather as a bilateral structure, we are met at once by the fact that the symmetry is by no means perfect. There is great range in the degree of bilateral asymmetry from its almost complete absence, as in certain neck hackles (Plate VIII, Figs. 58–60), to very pronounced asymmetry both of form and



FIGS. 9–12.—Drawings from photographs (Plate VI, Figs. 41–44); they show the development of the thyroxin mark (represented in black) in the saddle feather germ after the injection of 5 mg. of thyroxin.

FIG. 9.—Twenty-four hours after injection.

FIG. 10.—Forty-eight hours after injection.

FIG. 11.—Seventy-two hours after injection.

FIG. 12.—Ninety-six hours after injection.

of pigmentation, as in the wing primaries of the Brown Leghorn. The degree of the deviations from symmetry exhibits certain regular relations to position in a definite feather tract, and whether nearer to, or farther from, the mid-line of the entire body. We shall not, however, attempt to define these relations in any detail in the present paper.

The matter that concerns us here is that asymmetry means differences in growth-rate on the two sides of the feather germ at some time, or throughout development. On the principle of relation between the thresholds of reaction to homones and growth-rates, these

differences should be reflected in pattern, and conversely asymmetrical patterns should throw some light on growth-rates.

The differences in growth-rate that produce asymmetry do not involve any difference in the character of the growth gradient in the barbs with which we have been concerned, but rather a difference in level of the gradients as such. This is, indeed, the essence of the asymmetrical pattern, i.e., that the two sides of the vane may behave independently of one another—quite as much so as though they were separate feathers—by virtue of the level of growth-rate inherent in each. Thus, one side of the vane may have a growth-rate comparable, let us say, to the rate of a feather from the anterior breast, and the other side comparable to a feather from the posterior breast (see text Fig. 2).

The differences in growth-rate appear often, or usually, to concern the shaft as well as the barbs; and the consequence is that the shaft in such asymmetrical feathers shows a more or less pronounced curvature toward the more slowly growing side. The same side may also have a somewhat smaller number of barbs in the vane than the more rapidly growing side.

The female markings produced by an interrupted series of injections of female hormone in breast feathers of capons are often asymmetrical, and in such cases it is noticed that the larger female area is on the concave side of the shaft, thus corresponding to a slower growth-rate and lower threshold. Conversely, the reversionary male color is more largely developed on the convex side. Both the curvature and the asymmetry of markings are in reverse position with reference to the axis of the body in feathers taken from opposite sides of the body.

In some cases this condition becomes extreme. Figures 51 and 52, Plate VII, are photographs of the fifth in the series of wing covert feathers counted in proximal-distal direction in each wing from the right and left wings, respectively. The bird was injected with 400 rat units of female hormone on June 1, 1931, and again on June 2. The feathers had been regenerating since May 18, and the regenerated feathers were plucked for record on June 27. It will be observed that the female mark is confined to one side of the vane in each case: to the left side of the vane in 51 and the right side in 52.

If it were possible to hold a steady level of concentration of female hormone throughout regeneration, a complete bilateral gynandromorph feather would be produced, with reversed asymmetry on the two sides of the body. Such asymmetry of growth-rates on the two sides of the vane is the undoubted explanation of the bilaterally gynandramorph feathers described by Pézard, Sand, and Caridroit (1926) and by Bond (1913).

Asymmetry appears in early stages of the feather germ; thus, if the cross-section of a 9-day germ shown in Plate II, Figure 8, be examined, it will be seen that there are fifty-six ridges on one side of the dorso-ventral line, and only forty-eight on the other side. This seems rather extreme asymmetry to be characteristic of such relatively symmetrical feathers as those of the breast; but there is not the slightest reason to suppose this specimen to be in any degree abnormal. Until we have had more time to examine the subject with greater care, we do not wish to generalize, beyond pointing out the probability that asymmetry of the feather, as in the case of other developing structures (entire eggs, limb buds, otic vesicles), is undoubtedly of very early origin.

In our recent experiments we have taken great care to record each feather or germ as to its exact location with reference to the major axes of the bird's body. Numerous examples of reversed asymmetry have thus accumulated in our records. Some of these are recorded in Plate IV, Figures 20–25; Plate V, Figures 26 and 27; and Plate VII, Figure 49. We are intending to make the asymmetry of the feathers the subject of a special paper at an early date.

III. EXPERIMENTAL MODIFICATION OF FEATHER TYPE AND PATTERN

A. INTRODUCTION

The modifications to be considered are produced by subcutaneous injections of female hormone suspended in olive oil, and of Squibb's crystalline thyroxin dissolved in distilled water rendered slightly alkaline. Injection involves a period of increase of hormone concentration in the blood following injection, presumably less rapid when olive oil is the medium, and a period of fall of concentration

with cessation of injections, due to excretion of the hormone. The threshold of any resulting reaction, given an adequate dose, is thus more or less gradually attained, and then gradually lost. The nature of the markings obtained is dependent on the processes of absorption and excretion, the concentration attained, and on the threshold levels in the barbs.

The markings on the vane are dependent on the reactions of the individual barbs. The threshold of reaction is lowest at the base of each barb where it joins the rhachis, and highest at the apex of each barb, i.e., at the margin of the vane, with a gradient of threshold of reaction between. The slope of this gradient appears to be different in breast feathers from that in saddle feathers, for instance—a matter that will be discussed as we proceed.

Translated into terms of the growing feather germ, we note first that reactions are determined immediately above the growth centers (text Fig. 1), though probably at slightly different levels for different types of reaction, such as kind of pigment formed, or presence or absence of barbules; and second that the threshold of reaction rises from the dorsal to the ventral surface of the feather germ corresponding to the definitive levels of the barbs from base to apex.

The statements in the preceding paragraph are perfectly objective; but, when we attribute the descending gradient of thresholds along the barbs to a corresponding decrease of growth-rate, we are relying on the determinations of gradients of growth-rate described in section II. It will be seen in the present section that gradients of growth-rate and threshold gradients agree very accurately. We are also relying on the careful determination of Juhn, Faulkner, and Gustavson (1931) that the threshold of reaction of different feather tracts is a function of the varying rates of growth of the feathers belonging to these tracts. It is logical, and indeed necessary, to hold that this principle applies to the parts of a feather as well as to different feathers; the independent determination of gradients of growth in the individual barbs and their agreement with thresholds of reaction is hence a welcome extension of a principle already established. We shall accordingly feel free, in what follows, to equate threshold of reaction with growth-rate in respect to the individual barb.

B. EXPERIMENTAL

We shall deal with results obtained with two different kinds of hormones, the female sex hormone and thyroxin, on feathers belonging to three tracts principally: the breast, the saddle, and the neck. For our purpose the use of these substances is for analyzing the physiological foundations of feather form and pattern, and we shall not therefore devote particular attention to the specific physiological properties of the substances used. We need note only that the female sex hormone appears to be specific for reactions conditioning the female type of feather characteristic of each feather tract; in the absence of the female hormone, in the breed of fowl dealt with, the male type of feather develops. The use of the female hormone, if interrupted during growth of the feather, produces so-called "gynandromorph feathers," generally of the transverse type. In the present stage of our knowledge it is difficult to analyze this specificity farther, but for our purpose it can be accepted as very exact. Thyroxin, on the other hand, produces, in "physiological doses," no effect upon the breast feathers but, on the other hand, very marked effects, resulting in interesting new patterns, on growing saddle and neck feathers. Certain aspects of these effects may be similar to effects of the female hormone, as in causing extension of barbules in neck and saddle feathers, but other aspects are entirely different.

Our results will therefore be classified by the kinds of feathers dealt with, and we shall begin with the breast feathers.

1. THE BREAST FEATHERS

The breast feather is uniformly colored, black, in the male; in the female the feathers are salmon in color, or buff stippled with brown. The tip is rounded and the barbs bear barbules along their entire length. Breast feathers are the most rapidly growing contour feathers (Juhn, Faulkner, and Gustavson, 1931); and in the breast the rate of growth increases in an antero-posterior direction. Breast feathers in general have the highest threshold for the female hormone of all contour feathers, and in the breast itself the posterior feathers have a higher threshold than the anterior feathers. Gynandromorph

feathers in any combination of transverse barring may be produced by injections of female hormone regulated as to time and dosage. Incomplete female bars and female markings may be produced at lower concentrations of the female hormone or by brief periods of hormone action.

The pattern of the female modifications obtained in the male breast feather by single or discontinuous injections of the female hormone depends primarily on the differences in rate of growth of the individual barbs in the feather germ and the correlated thresholds of response described in the preceding pages.

Since these observations form the basis for the experimental results given here, they may be recapitulated in brief (refer to text Figure 1). The growing parts of the dorsal ridges, immediately adjacent to the rhachis, represent the extreme basal sections of the forming barbs. They have the lowest rate of growth and the lowest threshold of response for the female hormone. The growth-rate and, correlatively, the hormone threshold increase slowly and progressively in the ridges to the right and left of the rhachis. In the extreme ventral ridges the acceleration of the rate of growth becomes very marked; these ridges represent the extreme apical sections of the developing barbs and form the margin of the definitive feather.

These relations are so constant that the female pattern resulting from graded dosages of the hormone may be predicted once an effective concentration is empirically established for the bird (and feather area) in question.

However, another factor enters into the realization of the female pattern: this is the principle of reaction rate. The ventral ridges, which, as repeatedly stated, are the regions of most rapid growth, show female modification within 24 hours after the administration of heavy hormone dosages. But in the ridges with slow growth-rate, (basal sections of future barbs) female modifications do not appear until 48 or, more frequently, 72 hours after hormone administration, irrespective of concentrations employed.

The relations shown between growth rate and hormone threshold and between growth rate and reaction rate acting either singly or together during feather development, appear adequate for the explanation of the various feather patterns produced. These feather

patterns and the physiological basis for their manifestation are discussed in the following:

a) *Patterns in regions of low female hormone threshold; axial patterns.*—The modifications resulting from low hormone dosages (text Fig. 13) are limited to the regions of lowest growth-rate—viz., the rhachis and the immediately adjacent ridges of the germ; with

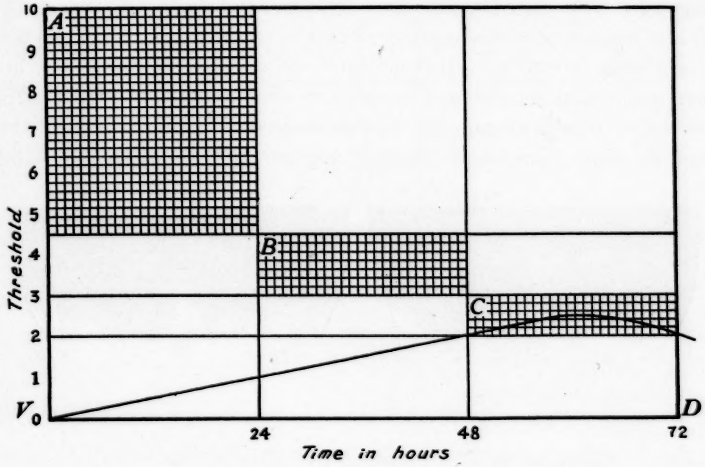


FIG. 13.—Reactions of breast feathers of capons to the female hormone. The amounts of reaction in 24 hours are represented by right-angled areas cross-hatched: A = ventral zone of the feather germ, corresponding to the margin of the definitive feather; B = intermediate zone of the feather germ, corresponding to the central part of the half vane; C = dorsal zone, corresponding to the axial part of the vane. These areas are arranged on a vertical axis to represent threshold requirements and on a horizontal axis to represent time required to give a visible reaction, i.e., to represent reaction rate. The curves projected on this surface represent rise and fall of concentration of female hormone according to injections given with reference to both threshold requirements and reaction rates. They agree with the experimental dosages for specific patterns.

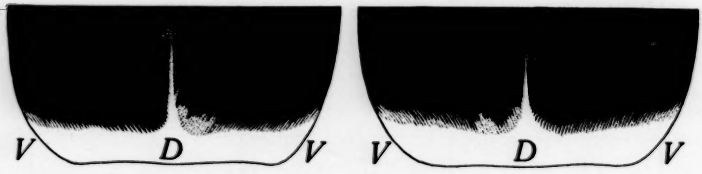
The base line represents the hormone level at the beginning of the experiment, in this case zero. D = dorsal surface of feather germ; V = ventral surface.

Patterns in regions of low female-hormone threshold, axial patterns.

very low dosages the effect is restricted here. If, however, the hormone concentration rises somewhat in the succeeding day or days, owing to successive injections, a number of ridges to either side also respond. Finally, with diminution of hormone concentration after

cessation of injection, again only the rhachis and adjacent ridges are modified. In the definitive feather the least reaction causes a female pattern practically restricted to the shaft and its immediate neighborhood with a rising and falling wave of reaction; a diamond-shaped or oval mark is formed with the shaft as long axis, thus recording the rise and fall of hormone concentration very clearly (Plate VII, Fig. 49).

The female response to dilute hormone concentrations reveals differences in rate of growth that must be very small. Such are, for instance, reactions confined to one side of the feather which are repeated in inverse asymmetry by the corresponding feather from the opposite side of the body (Plate V, Fig. 26 and 27; text Figs. 14 and



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FIGS. 14 AND 15.—Drawings from photographs (Plate V. Figs. 26 and 27; see for protocol) of feather germs from right and left sides of the breast. They illustrate the asymmetry of the female reaction (represented by stippling), exhibited by axial patterns.

15). It is clear that the rate of growth on one side of the vane has been above the threshold level, but below it on the opposite side; and the reversal of this relation on the opposite side of the body shows that we are dealing, not with an incidental, but with a constant relationship.

The original asymmetry of the feather germ has been discussed in Part II, section 5. It is clear that the threshold differences for the female hormone reflect this condition.

b) *Patterns in regions of all thresholds; bars.*—Female type bars result from hormone concentrations adequate for the satisfaction of all thresholds and thus entailing a response in the entire zone of reaction (text Fig. 16). The actual configuration of the individual

barred patterns varies, and the variations occurring are founded in the differences in hormone threshold and in reaction rate within the feather germ, discussed earlier.

Female hormone dosages of *initial and continued* high concentrations (text Fig. 16, curve *II*) cause the appearance of modifications first in the ventral ridges, since these require the least time for visible response. Thus, not only has this region an advance over the other ridges, but its own inherent most rapid growth-rate determines the

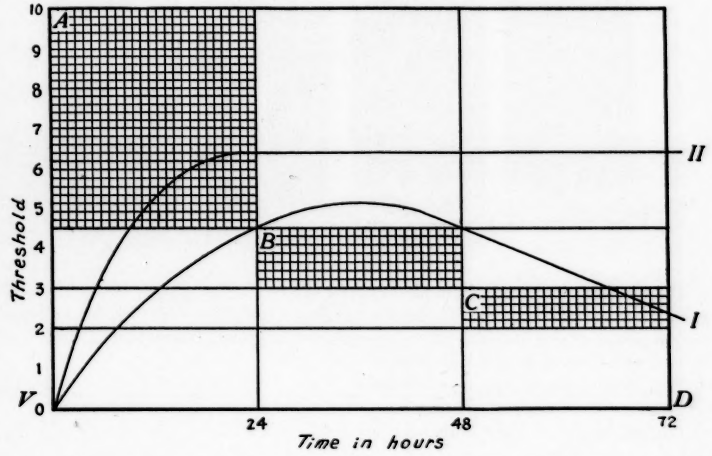


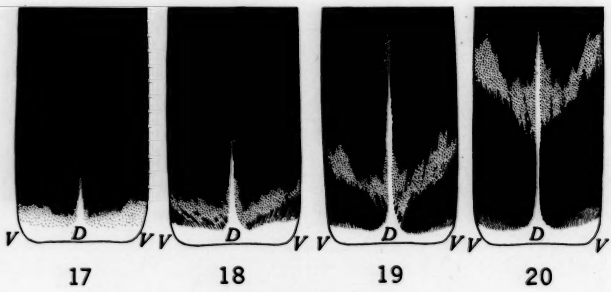
FIG. 16.—See figure 13. Patterns in regions of all thresholds; two types of bar (consult text).

formation of longer modified barb sections within given time intervals of maximum hormone action. In the definitive feather a female transverse bar so determined will therefore possess a wider marginal section (Plate VII, Figs. 47 and 48). The frequently encountered expansion again of the female bar near the shaft marks the continued response of the ridges near the rhachis during the gradual decrease of hormone concentration, when male reversion has already occurred in the other regions (text Figs. 7 and 8).

The female bars obtained with high initial hormone concentrations illustrate also, though in different manner, the asymmetry of the reaction in the feather germ. Here the asymmetry is apparent

in the difference in size of the marginal sections of the bar to the right or left of the shaft (Plate IV, Figs. 20–25). This reflects directly the difference in growth-rate of the right and left ventral ridges in the collar.

Female hormone dosages may also be so adjusted that a gradual rise and fall in concentration is effected (text Fig. 16, curve *I*). Such concentration curves determine different time intervals of re-



FIGS. 17–20.—Drawings from photographs (Plate V, Figs. 28–31; see for protocol). They show the development of a female bar (represented by stippling) in the breast-feather germ and also illustrate the more rapid rate of growth in the ventral zone.

FIG. 17.—Two days after injection.

FIG. 18.—Three days after injection.

FIG. 19.—Four days after injection.

FIG. 20.—Six days after injection.

action in the various regions. Stated somewhat diagrammatically: The threshold requirements of the dorsal ridges are satisfied before the curve of concentration achieves the peak essential to modification in the ventral ridges. Now, as shown, the regions of high threshold require less time, after the threshold requirement is satisfied, for a visible response than do the regions of low threshold. Therefore the interplay of these two reaction modes may be so balanced as to determine a simultaneous visible response in all regions, i.e., the first appearance of female modification is a band parallel to the reaction zone in the entire collar (Plate V, Figs. 28–31; text Figs. 17–20). With gradual fall in concentration male reversion of course occurs earlier in the ventral than in the dorsal ridges. The regions of low threshold thus continue development under female hormone in-

fluence for a longer period. However, this *longer* continued growth in the dorsal section under the influence of the female hormone is compensated in the ventral section by a more *rapid* growth during the briefer period of hormone effect; the definitive female bar thus possesses a relatively uniform width over its entire extent.

When the rise in concentration of the female hormone to the degree essential to modification in the ventral ridges, and the subse-

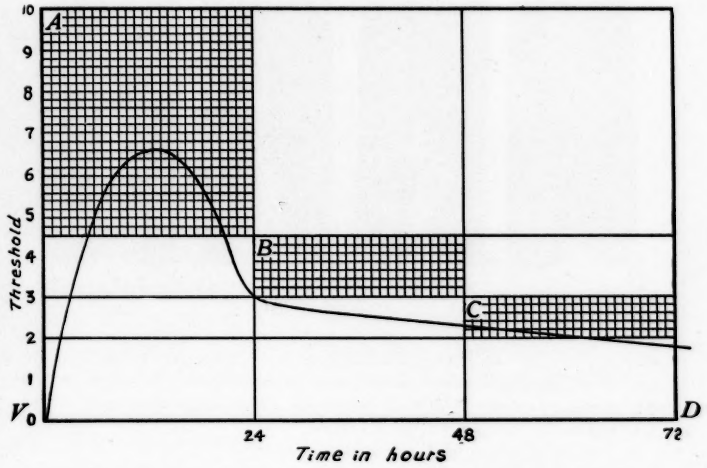


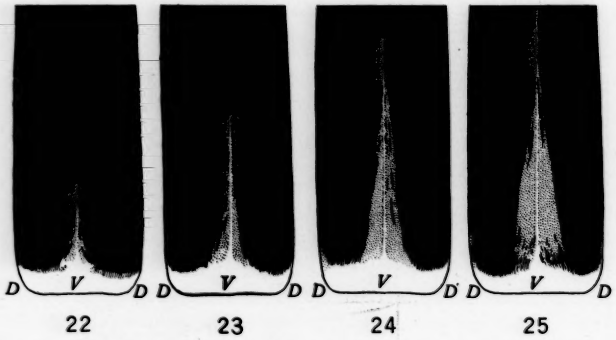
FIG. 21.—See Figure 13. Patterns in regions of high female-hormone threshold; marginal patterns.

quent fall, is very gradual, the longer female growth period thus assured the regions of low threshold outweighs the more rapid growth in the regions of high threshold during the brief period of female hormone concentration adequate here. The female bar in the definitive feather so determined has a wide central region tapering laterally to narrower marginal zones.

c) *Patterns in regions of high female hormone threshold; marginal patterns.*—Very high female hormone concentrations persisting for sufficiently brief periods would satisfy time and threshold requirements of the regions of most rapid growth-rate only (text Fig. 21). Female modifications with such dosages should be restricted to the

ventral region of the feather germ and appear only in the margin of the definitive feather (Plate VII, Fig. 50).

Rapid resorption follows the injection of single, very massive doses, but excretion is not continued directly to the zero point and some residual hormone persists for a number of days. Female modification occurs in the feather germ as to be expected with such dosages first in the ventral ridges; however, traces of female modifica-



FIGS. 22–25.—Drawings from photographs (Plate V, Figs. 32–35; see for protocol). They are ventral preparations of feather germs from the breast to show the development of a marginal pattern. The female reaction is indicated by stippling.

FIG. 22.—Twenty-four hours after injection.

FIG. 23.—Forty-eight hours after injection.

FIG. 24.—Seventy-two hours after injection.

FIG. 25.—Ninety-six hours after injection.

Note in Figures 24 and 25 the appearance of female reaction in the rhachis, previously confined to the ventral region.

tion may appear later also in the extreme dorsal ridges and rhachis, determined by the residual hormone traces persisting for the period necessary to modification in this region (Plate V, Figs. 32–35; text Figs. 22–25). The intermediate ridges remain entirely unaffected, showing that the concentration of the hormone diminishes so rapidly that adequate amounts did not persist over the time interval essential for female modification here.

Plate V, Figures 32–35, and text Figures 22–25 show the first appearance of female modification and the time of its persistence in the ventral ridges during 4 days. On the fourth day when reversion is

setting in ventrally (Plate V, Fig. 35; text Fig. 25) female modification in the rhachis and immediately adjacent ridges also appears. It may be noted that the feather germ preparations in this case are split along the rhachis since the ventral zone is of greatest interest here; the ventral zone forms the center, and the longitudinally bisected rhachis the margins, of these preparations. Figure 50, Plate VII, shows the pattern formed in the definitive feather in such an experiment.

2. THE SADDLE FEATHERS

The feathers of the saddle exhibit a high degree of sexual dimorphism. In the female the feathers are round-tipped and shaped much like breast feathers with barbules extending the entire length of each barb. The feathers are finely and evenly stippled, dark brown on brown. In the male the homologous feathers are acuminate, much longer than in the female and lacy at the tip and along the margins due to the absence of barbules, which are confined to the region of the shaft anteriorly but in a posterior direction gradually spreading laterally from the shaft, so as to produce a central, acuminate web. The color is a brilliant orange; the web is somewhat darker throughout, and in its base there is a region of very fine dark stippling asymmetrically placed with reference to the shaft.

The saddle feathers of the male thus offer a wider range for modification than do the breast feathers, in which only pigment changes occur with female hormone. The male saddle feather differs from that of the female in pigment, absence of barbules for a large part of its area, and in outline. The feather outline varies in general with barbule formation; barbule and pigment formation, however, while definitely associated in the normal feather, may be separated experimentally.

In the present study the effects of subcutaneous injections of Squibb's crystalline thyroxin form the basis for analysis. The patterns produced by injections of female hormone are also introduced later for comparison. Thyroxin causes a quantitative series of changes in the feather germ and pin feather, increasing in the order of concentration. It should be stated that the doses of thyroxin used were, in all the experiments recorded here, so adjusted that, save in one instance (10 mg.), molting did not occur; and the induced

plumage changes were restricted to saddle and neck feathers, which are the lightest-colored feathers of the entire plumage.

Following the injection of thyroxin, a blanching of pigment already laid down occurs in the barbs surrounding the pulp; this takes place within 24 hours; it is simply a dilution of the color already present to a lighter tone, and is not to be confused with effects produced in the growing zone. In fact, this blanching follows extremely low dosages and may occur entirely alone. It becomes more pronounced with stronger concentrations, always forming the tip of an induced thyroxin mark. Compare Plate VIII, Figures 53–57.

With increasing thyroxin concentration, the second response in the series appears; in regions normally free, barbules are formed. Finally, when the dosage is even more concentrated, black pigment appears.

Blanching, barbule formation, and pigment deposit follow each other in order of increasing thyroxin concentration. Blanching may occur alone; but with barbule formation blanching always takes place; and pigment formation is always associated with both blanching and barbule formation. These several steps in the feather reaction to rising concentrations thus offer a method of estimating concentration curves, and the pigment deposition may serve as an indicator for thyroid extracts. The possibilities of this are discussed by Juhn and Barnes (1931) and are being further investigated.

The physiology of development of the feather germ is most readily studied with doses adequate for pigment deposit, since the black granules outline the reaction zone affected in brilliant contrast to the unmodified, orange barb sections. The visible response to single doses of thyroxin was found to persist always at least 4 days, irrespective of initial dosage.

As in the breast, the ventral ridges of the saddle feather germ are the regions of most rapid growth, and rate of growth in the individual barb centers diminishes progressively toward the rhachis (cf. p. 172).

As in the case of the breast feathers, patterns in process of formation in the germ and the definitive resulting patterns are compared. The technique is the same: plucking areas to have feather germs of the same time of regeneration for comparison, injections at stated

times, plucking and preparation of germs at stated periods after injection, and preservation of definitive feathers of the same series.

Dilute concentrations of thyroxin result in modifications of the dorsal ridges only, the region of least growth rate and lowest threshold. Text Figure 26, curve *I*, and Plate VI, Figures 36–40, show feather germs prepared at daily intervals after the injection of 0.5 mg. of thyroxin. The first response, limited to the rhachis and immediately adjacent ridges, occurs after 48 hours; it is continued in approximately the same degree for about 4 days, when reversion occurs (text Figs. 27–30). In the definitive feather (Plate VIII, Fig. 53) the pattern so determined is a very narrow spindle practically restricted to the shaft. The widening of such a mark from top to bottom is a measure of the progressive rise and fall in the concentration of the thyroxin; the extension of the long axis records the time effectiveness.

With increasing concentration of the single thyroxin doses administered, the patterns produced become progressively more diamond-shaped, i.e., the transverse axis extends farther across the feather, showing that an increasing number of ridges to the right and left of the rhachis were involved, until finally a response is induced in the entire zone of reaction and the mark extends across the entire width of the feather (cf. text Fig. 26). Plate VIII, Figures 53 and 57, are saddle feather patterns showing the gradual passage of spindle to bar resulting from single doses of thyroxin of increasing concentration.

In examining the series of these marks it will be noticed that the anterior end gradually straightens out transversely but that the posterior end does not. This measures the sudden rise of concentration of thyroxin in the blood after injection and its much more gradual elimination. It will also be noted that even with the highest concentration a pointed tip of pigment projects from the center of the anterior boundary along the rhachis; this measures the lower threshold of the extreme basal section of the barbs. The inverted triangle shape of the markings produced by relatively high doses comes out particularly well in a series of spaced injections, as illustrated for the neck feathers in Plate VIII, Figures 59–60.

The developmental history of these markings is shown in Plate

VI, Figures 36–40, for 0.5, and Figures 41–44 for 5 mg. of thyroxin. The relations between these and the definitive markings will be readily understood on the basis of principles already stated. The series of stages from birds receiving a 5 mg. injection (Plate VI, Figs. 41–44; text Figs. 9–12) gives visible proof of the growth gradient in the barbs, for the anterior boundary of the mark begins as a transverse line (41) and is gradually converted into a *V* (44) by the more rapid growth of the ventral ridges. The slope of the *V* to the

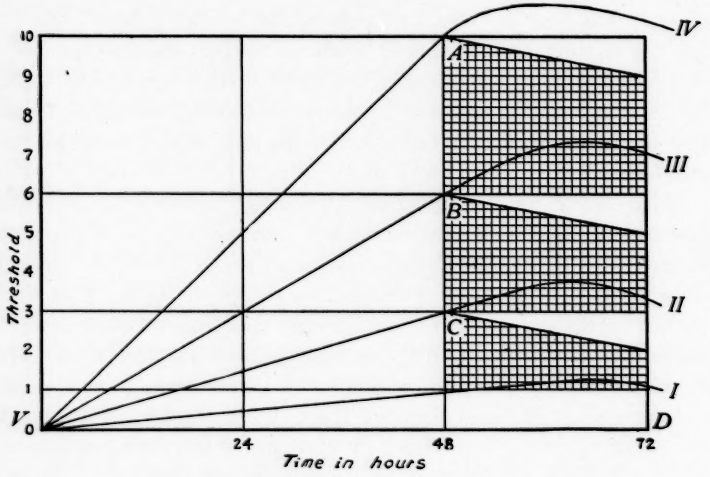


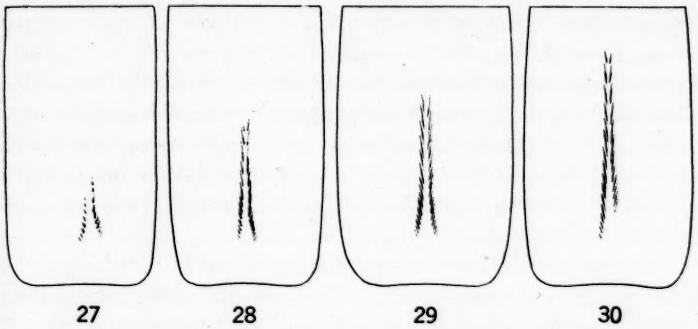
FIG. 26.—Diagram of thyroxin patterns in the saddle feather. Curve I = 0.5 mg. of thyroxin; curve II = 1.0 mg. of thyroxin; curve III = 1.5 mg. of thyroxin; and curve IV = higher doses of thyroxin.

The areas *A*, *B*, and *C* represent approximately the quantity of reaction in the marginal, central, and axial regions of the vane in 24 hours, 48 and 72 hours after injection, owing to gradation of growth-rate along the axes of the barbs. The curves represent the intersection of thresholds at various concentrations as described in the text.

Curve I, when completed by excretion of excess thyroxin, will produce a spindle-shaped axial mark (Plate VIII, Fig. 53); curve II, a broader spindle or diamond (Plate VIII, Fig. 54); curve III (Plate VIII, Fig. 56), an inverted isosceles triangle extending at its base completely across the vane; curve IV, a similar triangle varying to greater density of pigmentation with basal angles transected.

point where the limbs join the rhachis extension is equivalent to a curve of growth-rates of the successive segments of the barbs.

Repeated injections of thyroxin at suitably spaced intervals result in a seriation of patterns along the entire long axis of the definitive feather. Such repeated dosages often result in a rapid molt and replacement of feathers, especially in the neck. The resulting plumage patterns are extremely interesting; with low dosages, the modified region is restricted to the vicinity of the shaft, leaving an unmodified margin and a beaded axial pattern (Plate VIII, Fig. 59); with high dosages, the successive modified areas extend to the extreme



FIGS. 27–30.—Drawings from photographs (Plate VI, Figs. 37–40) of saddle-feather germs showing the development of a thyroxin mark (represented in black) resulting from a 0.5 mg. injection.

FIG. 27.—Forty-eight hours after injection.

FIG. 28.—Seventy two hours after injection.

FIG. 29.—Ninety-six hours after injection.

FIG. 30.—One hundred and twenty hours after injection.

edges of the feather at their anterior borders, tapering thence to the anterior border of the next marking, leaving between a series of unmodified marginal areas which constitute a "negative" marginal pattern (Plate VIII, Fig. 60).

The more apical beads of such a series of marks due to successive injections of identical quantities of thyroxin are more heavily pigmented than less apical beads of the same series. This may be seen in Figure 60, Plate VIII, though the photograph does not do full justice to the contrast. Single marks also in separate feathers exhibit the same pigment gradation when occurring at different levels. This is a perfectly constant phenomenon exhibited also with repeated

doses of female hormone (cf. Fig. 13 of Juhn, Faulkner, and Gustavson, 1931). Barbule formation, which occurs at a lower threshold, is not so much affected. There are obviously slight physiological differences along the axis, which result in striking morphological effects with the same hormone concentration. The results seem to imply a slightly higher level of the growth gradient in the more basal barbs.

As mentioned earlier, the saddle feathers of the Brown Leghorn show a region of dark pigment at the base of the vane, and this is asymmetrical, often restricted, either entirely or for the most part, to one side of the rhachis, the position varying according to the origin of the feather from the right or left side of the bird's body. It appears that the induced thyroxin patterns repeat this original asymmetry in the effects produced at the base of the vane. Like the patterns induced with the female hormone in breast feathers from opposite sides of the body, these effects also exhibit reversed asymmetry in feathers from right and left sides.

The distribution of symmetry relations in normal feather patterns is an extremely interesting subject in itself, and it has received careful attention for spangled breeds of fowl by Landauer (1930).

The response of the saddle feather to the female hormone has been studied only in the definitive feather; and here we possess numerous observations, largely unpublished, both on continuous and intermittent injections of different hormone concentration. The feather germ reaction forming the basis for these female modifications may readily be interpreted from their pattern since the mechanism of response has been determined.

Inasmuch as the female saddle feather differs from the male in the possession of barbules in the entire feather surface as well as in the pigment, the reaction of the feather germ to the female hormone involves two components instead of one only, the pigment, as in the breast feather.

In the order of response to increasing female hormone concentrations, barbule formation is obtained first; the modified feather or feather section may retain the orange pigment in the entire area. Such barbule patterns behave in accordance with the general princi-

ples recorded for thyroxin reactions; with very low dosages, they occur near the rhachis; with increasing dosage, they extend toward the feather margin. When the dosage is sufficient to insure the response of all the ridges in the feather germ, the hormone concentration appears usually to have achieved the level essential for pigment modification, and the specific female pattern and colors appear. Hormone concentrations slowly rising to levels adequate for complete female modification, and then gradually decreasing again, produce diamond- or bead-shaped patterns having an anterior and a posterior barbule-bearing margin and an entirely female median section.

The saddle-feather response to dilute female hormone dosage produces patterns almost indistinguishable from those obtained with low doses of thyroxin because the formation of barbules has a low threshold for each. However, there is no question of identity of action of the two hormones, for, when the entire plumage is considered, even dilute dosages of female hormone will cause the appearance of salmon pigment in breast feathers in regions of slow growth-rate (in the anterior breast, near the throat), and this does not occur with thyroxin in any concentration. Moreover, the final steps in saddle-feather response, viz., the entirely female feather produced by adequate concentrations of female hormone, and the abnormalities produced by heavy doses of thyroxin are utterly different.

The function of the female hormone is to set free, to unlock, the potentiality for female development resident in the constitution of the feather germ. The hormone exteriorizes the morphological and physiological characteristics of the female cell; but both these are the attributes of the *cell*, determined in their entirety and only dependent on the hormone for expression.

If the female hormone appears to increase the metabolic rate in the saddle feather, it is because the growth-rate of the female-induced saddle feather is more rapid than that of the male of the same region. In general its effect on rate is to bring about correspondence with the normal female rate, whether this be greater, as in the saddle, or less, as in the breast, than the male.

IV. DISCUSSION: GROWTH-RATE AND HORMONE THRESHOLD

Growth-rate has been related to the form of an organism or parts thereof, in certain obvious direct ways, such as are associated with the principle of unequal growth in embryonic development, from the time of His (1874) down to the present day. Differences in proportions of parts, changes in relative positions, outfolding and infoldings, etc., are attributable to such differences of growth-rate regularly distributed as to sequence in time and to location in the embryo. The disposition of regularly graded growth-rates, or rates of metabolism, along axial systems, as in Child's theory of the axial gradient, have also played a considerable part in recent times in physiological theories of development; from this has arisen the idea of "dominance" of the most active part of such a system. Growth-rate has also been studied as a function of the age of the organism; as affected by temperature and other physical conditions; as related to physiological epochs such as puberty or metamorphosis or regeneration and repair; etc. The conception that genes operate in large part through their effects on growth or developmental rates is also a leading idea in Goldschmidt's "physiological theory of heredity" (1927) and is the subject of Ford and Huxley's study on "Mendelian Genes and Rates of Development in Gammarus" (1927). In short, the distribution of growth-rates in the organism, whether at different epochs of the life-history or in different parts of the body, is a very old and much-studied subject.

The relation between growth-rate and hormone threshold is, however, a new aspect of the matter first clearly stated by Juhn and Gustavson (1930). Pézard (1922*a* and 1922*b*), it is true, applies his idea of differential threshold and the law of "all-or-nothing" to the explanation of gynandromorphism in birds. He points out that the threshold of reaction to the sex hormones is not exactly the same for all characters. This he determines by equating the size of fragments of the testis remaining in operated birds with the sex characters developed, and also by determining the order of appearance of sex characters in normal development which is assumed to depend on the rising flood of hormone development. Zawadowsky (1926) has noted in addition a difference in threshold of reaction to the female hormone in different feather tracts. Neither

of these authors, however, has related the differences in threshold to rates of growth. It is obvious that this principle may have a rather wide application in growing systems in general, whether in the embryo or in the adult; and considerable ingenuity might be exercised in attempting to apply it to various embryological, genetic, and physiological events wherever symmetries exist in animals or plants. We shall not, however, attempt to go beyond its applications to certain phenomena exhibited by plumage in birds.

In the case of the feather germ, its specific potency as feather germ, its genetic composition as belonging to a definite species and variety and to a definite feather tract of that variety, are given conditions in physiological experimentation. So also are the distribution of growth-rates, and the order of the origin and the nature of the fundamental segregates belonging to each specific germ, such as the rhachis, the barbs, the barbules, and the chromatophores. The main thesis of this paper is that general physiological conditions produce morphological diversity in genetically identical feather germs, and that the main cause of the diversity of action is definitely oriented, accurately regulated differences of growth-rate within the germ. This leaves a wide range of form and pattern for experimental modification.

The differences in growth-rate that we have studied relate to differences between different feather tracts (Juhn, Faulkner, and Gustavson, 1931), to graded differences of growth-rate along the axes of the barbs, and to differences between the two sides of the vane of the same feather (bilateral asymmetry).

Wherever constant differences of these kinds are found to exist, it is also found that there is a direct relation between the rate of growth and susceptibility to modification of the succeeding processes of differentiation: for any given process of differentiation determined by hormone action the threshold of its emergence in response to the hormone is directly proportional to its rate of growth.

Slowly growing feathers react to a smaller dose of hormone than rapidly growing feathers. As rate of growth is a fixed property of the different feather tracts, though subject to gradation within each tract, it is possible to produce birds, by means of suitable administration of female hormone, with female feathers in the slowly grow-

ing tracts, and feathers of male character in the more rapidly growing tracts. Numerous variations on this theme become possible by interrupted injections of female hormone during regeneration of feathers (Juhn, Faulkner, and Gustavson, 1931).

Similarly, when we know the rates of growth of the constituent parts of the individual feather, it is possible to interpret patterns produced by injections of female hormone or thyroxin with reference to gradients of growth-rate, as described in detail in the present paper.

Finally, differences in growth-rate, and consequently of hormone threshold, on the two sides of the vane furnish the explanation of longitudinal gynandromorph feathers such as have been described by Pézard, Sand, and Caridroit (1926) and by Bond (1913). The senior author (1931) has also shown how it is possible on the basis of the relation between hormone threshold and growth-rate, to explain the puzzling cases of bilateral gynandromorphs in birds as due to a combination of bilateral hemihypertrophy and ovarian deficiency (Lillie, 1931.)

For the present the relation between growth-rate and threshold of reaction is presented only as an experimentally determined principle. We have not considered in this paper the general physiological interpretation of the relationship between low rate of growth and low threshold of reaction, and between high rate of growth and high threshold. This matter requires further consideration.

V. SUMMARY

A. DEVELOPMENT OF THE FEATHER

The breast and saddle feathers of Brown Leghorn fowl were studied in the process of regeneration in the endeavor to project the elements of the feather back upon the germ. Special attention has been paid to these points:

1. The seriation of the barbs in development.

2. The rate of formation of barbs; number arising per day; time required to complete a barb.

3. The disto-proximal order of formation and differentiation of the individual barb.

4. The problem of growth gradient in barbs.

5. The relation of the shaft to the barbs during development.

6. The problem of asymmetry of the feather.

7. The localization of determination of the morphological elements and pigmentation of the barbs and shaft with reference to time and place in the feather germ—in short, the projection of the feather back upon the germ.

B. THE PHYSIOLOGY OF FEATHER PATTERNS

The experiments under this head demonstrate that the form of experimentally determined patterns depends on two principles:

1. The threshold of reaction measured by dosage is directly proportional to the growth-rate, low dosages producing axial patterns and high dosages being required to produce bars or marginal patterns.

2. The principle of reaction rate. The time required for realization of a reaction is proportional to rate of growth. Regions of high growth-rate record the hormone reaction, given adequate concentrations, more rapidly than the regions of low growth-rate. Since the apex of the barbs has the highest growth-rate, this establishes the possibility of producing marginal patterns.

3. With rising and falling concentrations due to the method of injection the time available for reaction diminishes from base to apex of the barb owing to rise by absorption and fall by excretion after cessation of injections.

4. The hormones used in these experiments were the female sex hormone prepared by Professor R. G. Gustavson and Squibb's crystalline thyroxin; the principles established hold for both.

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EXPLANATION OF PLATES

All figures are microphotographs and photographs from preparations of Brown Leghorn fowl.

PLATE I

FIG. 1.—Longitudinal section of feather papilla and part of the follicle immediately after plucking, from the breast. In order to avoid the presence of blood in the cavity of the follicle, the bird was killed and thoroughly bled. The feather was then plucked, and the skin containing the follicle cut out and sectioned. The outer surface of the skin is above with reference to the orienta-

tion of the figure. The upper surface of the papilla will therefore become the dorsal surface of the feather germ. Note absence of epidermis at apex of papilla, and blood vessel in its axis at the base.

FIG. 2.—A feather germ from the breast 8 days after plucking, ×11, photographed from the dorsal surface. At this time the feather germ has not yet grown to the mouth of the follicle. The preparation was made by dissecting the germ out of the skin after fixation; a small piece of skin still adheres to its ventral surface. *s*=sheath of feather germ; *a*=apex of pulp; *r*=ridges, forming barbs; *b*=base of feather germ including collar and umbilicus; *x*=first indication of rhachis.

FIG. 3.—Part of same preparation as Figure 2, ×30.

FIG. 4.—Longitudinal sagittal section of feather germ of same series as Figures 2 and 3. Breast, 8 days. ×18. *D*=dorsal surface; *V*=ventral surface; *a*=position of apex of dorsal barbs; *b*=approximate location of base of barbs; *f*=cavity of follicle; *u*=umbilicus; Fig. 5=part shown more highly magnified in Figure 5; Fig. 6=part shown more highly magnified in Plate II, Figure 6. Observe dense growing region of pulp within the umbilicus, and looser texture above, vascularity, etc. The epidermal portion of the germ is solid black at this exposure selected to bring out the structure of the pulp; note that the dorsal epidermal part is much thicker than the ventral part, owing to greater age of barbs dorsally than ventrally.

FIG. 5.—Region of umbilicus of Figure 4, ×70. *c*=cells of collar; *s*=feather sheath, cornification beginning; *e*=epidermis of follicle; *D*=dorsal; *V*=ventral.

PLATE II

FIG. 6.—Region indicated in Plate I, Figure 4, ×500. Longitudinal section of a ridge a very short distance above the zone of determination; for its exact location with reference to the total length of the ridge compare points *a* and *b* in Figure 4. *b*=barbules; *c*=chromatophores; *p*=pulp; *r*=total depth of ridge; *s*=feather sheath.

FIGS. 7–10.—Transverse sections through a 9-day feather germ from the right anterior breast. *b*=barbule cells, *c*=chromatophores; *f*=wall of follicle, *f.c.*=cavity of follicle; *p*=pulp; *r*=rhachis; *s*=sheath cells; *D*=dorsal; *V*=ventral.

FIG. 7.—Through the collar in the region of the barb centers. ×70.

FIG. 8.—Immediately above the collar through the unpigmented bases of the ridges. ×70.

FIG. 9.—0.61 mm. in front of Figure 8. ×70.

FIG. 10.—Ridges indicated in Figure 9. ×550.

PLATE III

FIGS. 11–19.—Split preparations of feather germs; these are prepared by splitting the wall of the feather germ along either the mid-ventral or mid-dorsal lines, removing the pulp and spreading out flat and fixing on a microscope slide.

If the split is ventral, the dorsal side is in the center, and vice versa. They are distinguished as "dorsal" and "ventral" preparations. The lower edge is formed by the mouth of the umbilicus. The unpigmented band includes the collar and the bases of the ridges.

FIG. 11.—Ten-day feather germ, left anterior breast. Dorsal preparation. ×5.

FIG. 12.—The base of the shaft and region of concrescence in Figure 11. ×50.

FIG. 13.—Ten-day feather germ, left anterior breast. Ventral preparation. ×5. *VT* = ventral triangle.

FIG. 14.—The region of the ventral triangle of Figure 13. ×50.

FIG. 15.—Twelve-day feather germ, left anterior breast. Dorsal preparation. ×5.

FIG. 16.—Twelve-day feather germ, left anterior breast. Ventral preparation. ×5. *T* = ventral triangle.

FIG. 17.—The region of the ventral triangle of Figure 16. ×50.

FIG. 18.—Fourteen-day feather germ, left saddle. Dorsal preparation. ×5.

FIG. 19.—Fourteen-day feather germ, left saddle. Ventral preparation. ×5.

PLATE IV

FIGS. 20–25.—Split and spread dorsal preparations of feather germs from right and left sides of the breast to illustrate reversed asymmetry of female reaction on the two sides.

FIG. 20.—Right anterior breast, 72 hours after the first injection.

FIG. 21.—Left anterior breast, 72 hours after the first injection.

FIG. 22.—Right anterior breast, 96 hours after the first injection.

FIG. 23.—Left anterior breast, 96 hours after the first injection.

FIG. 24.—Right anterior breast, 120 hours after the first injection.

FIG. 25.—Left anterior breast, 120 hours after the first injection.

Protocol. Capon 29–970. Feathers plucked 4-8-31 from four extreme lateral areas of the breast, left and right, anterior and posterior; injected with 500 rat units of female hormone on three successive days: 4-22-31, 4-23-31, and 4-24-31.

PLATE V

FIGS. 26–27.—Split and spread dorsal preparations of feather germs from right and left sides of the breast to illustrate asymmetry of female reaction on the two sides.

FIG. 26.—Right anterior breast, 72 hours after the injection.

FIG. 27.—Left anterior breast, 72 hours after the injection.

Protocol. Capon 29–35. Areas plucked 3-13-31, from corresponding extreme lateral right and left sides of the breast. Two weeks later injected with 1,600 rat units of female hormone (capon found by previous tests to be extremely resistant).

FIGS. 28–31.—Split and spread dorsal preparations of feather germs from the breast to show development of a female bar.

FIG. 28.—Two days after injection.

FIG. 29.—Three days after injection.

FIG. 30.—Four days after injection.

FIG. 31.—Six days after injection.

Protocol. Capon 29–941. Eight hundred rat units of female hormone injected 18 days after plucking anterior breast.

FIGS. 32–35.—Split and spread ventral preparations of feather germs from the breast to show the development of a marginal marking. The germ was split along the rhachis, and the ventral surface of the germ is therefore in the center.

FIG. 32.—Twenty-four hours after injection.

FIG. 33.—Forty-eight hours after injection.

FIG. 34.—Seventy-two hours after injection.

FIG. 35.—Ninety-six hours after injection.

The female reaction is at first confined to the ventral part of the germ, as shown in Figures 32 and 33; it then appears at the rhachis, Figures 34 and 35, skipping the intermediate region completely. See text, p. 168; for curve see text Figure 21. For definitive condition compare Plate VII, Figure 50.

Protocol. Capon 29–970. 4-29-31. Feathers plucked from posterior breast, extreme left lateral margin. 5-9-31. Bird injected with 1,500 rat units of female hormone.

PLATE VI

FIGS. 36–40.—Split and spread dorsal preparations of feather germs from the saddle to show the development of the thyroxin mark after injection of 0.5 mg. of thyroxin (cf. Plate VIII, Fig. 53).

FIG. 36.—Before injection.

FIG. 37.—Forty-eight hours after injection.

FIG. 38.—Seventy-two hours after injection.

FIG. 39.—Ninety-six hours after injection.

FIG. 40.—One hundred and twenty hours after injection.

Protocol. Capon 29–941. Feathers plucked between 1-29-31 and 2-12-31; 0.5 mg. of thyroxin injected 3-13-31.

FIGS. 41–44.—Split and spread dorsal preparations of feather germs from the saddle to show the development of the thyroxin mark after injection of 5 mg. of thyroxin (cf. Plate VIII, Fig. 62).

FIG. 41.—Twenty-four hours after injection.

FIG. 42.—Forty-eight hours after injection.

FIG. 43.—Seventy-two hours after injection.

FIG. 44.—Ninety-six hours after injection.

Protocol. Capon 29–941. Feathers plucked 2-26-31; 5 mg. of thyroxin injected 3-24-31.

PLATE VII

FIGS. 45–52.—Female patterns in definitive feathers.

FIGS. 45–48.—Bars (Protocol: cf. Plate IV, Figs. 20–25, and legend).

FIG. 45.—Right anterior breast feather.

FIG. 46.—Left anterior breast feather.

FIG. 47.—Right posterior breast feather.

FIG. 48.—Left posterior breast feather.

FIG. 49.—Axial marks in breast feathers. Note asymmetry of markings.

Protocol. Capon 29–62. 2-18-30. Feathers plucked. Injected with 300 rat units of female hormone on three successive days: 3-1-30, 3-2-30, and 3-3-30. Injections with 300 rat units of female hormone repeated on three successive days: 3-7-30, 3-8-30, and 3-9-30.

FIG. 50.—Marginal female area combined with axial female area in breast feather (Protocol: cf. Plate V, Figs. 32–35, and legend; see also text, page 167, and curve of text Fig. 21).

FIGS. 51–52.—Female marks limited to one side of the vane. The feathers are from opposite sides of the body, and the female patterns exhibit reversed asymmetry.

Protocol. 5-18-31. Feathers plucked. 6-1-31 and 6-2-31, bird injected on successive days with 400 rat units of female hormone.

FIG. 51.—The fifth feather in the series of right wing coverts in proximal-distal direction.

FIG. 52.—The fifth feather in the series of left wing coverts in proximal-distal direction.

Number 1 in each series is the most proximal feather to the mid-line of the body, respectively.

PLATE VIII

FIGS. 53–57.—Tips of saddle feathers recording increasing single doses of thyroxin.

FIG. 53.—0.5 mg.

FIG. 54.—1.0 mg.

FIG. 55.—1.5 mg.

FIG. 56.—5.0 mg.

FIG. 57.—10 mg.

FIGS. 58–60.—Neck hackle feathers recording repeated doses of thyroxin.

FIG. 58.—Normal control.

FIG. 59.—Bird injected with 1.0 mg. of thyroxin every seventh day.

FIG. 60.—Bird injected with 1.5 mg. of thyroxin every sixth day.

PLATE I

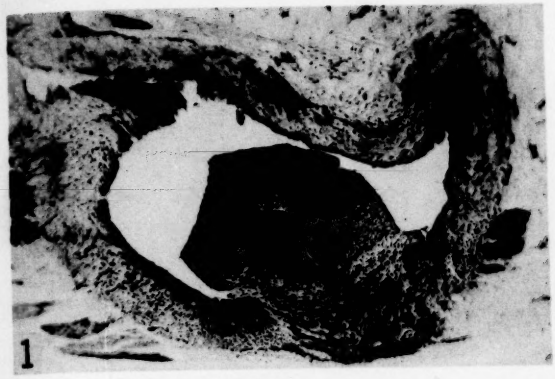
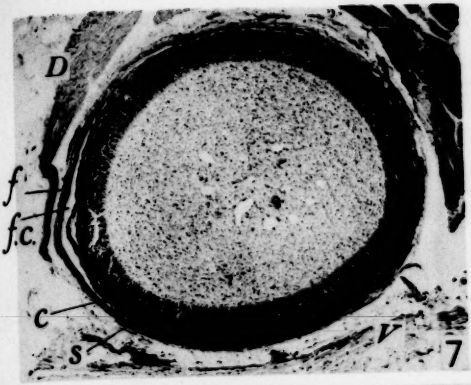
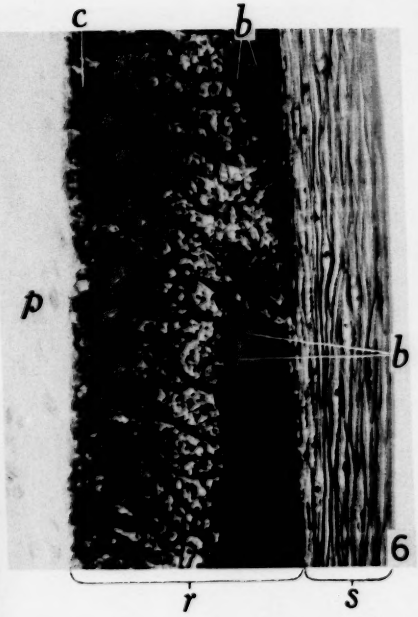
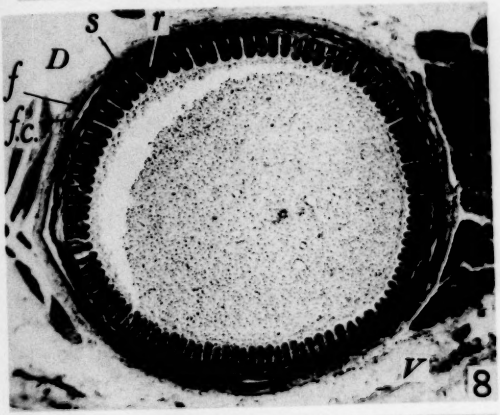
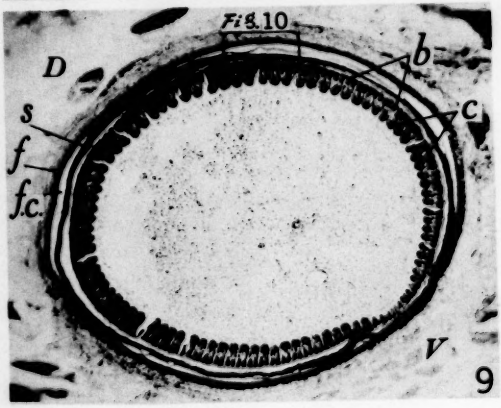


PLATE II









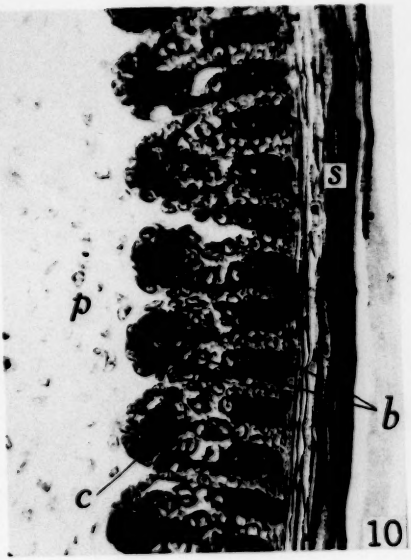
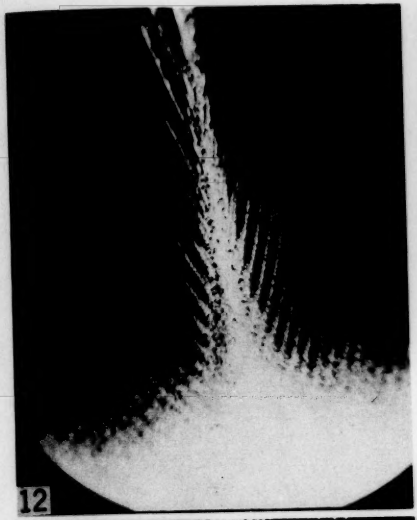


PLATE III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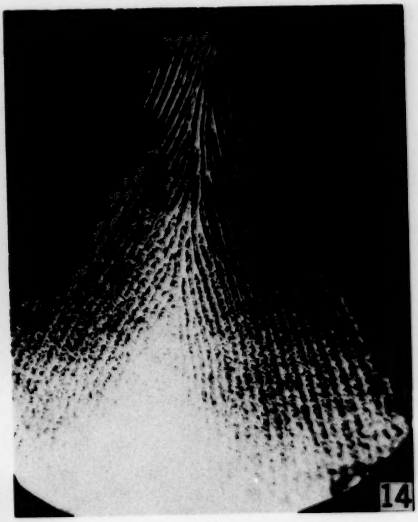
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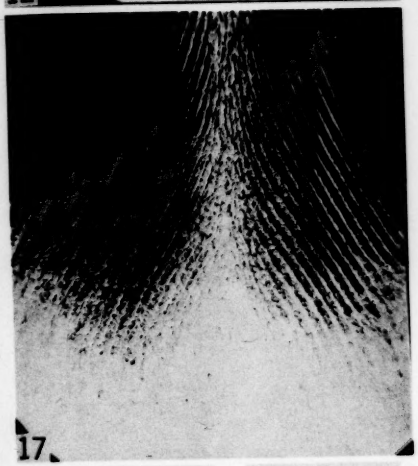
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PLATE IV

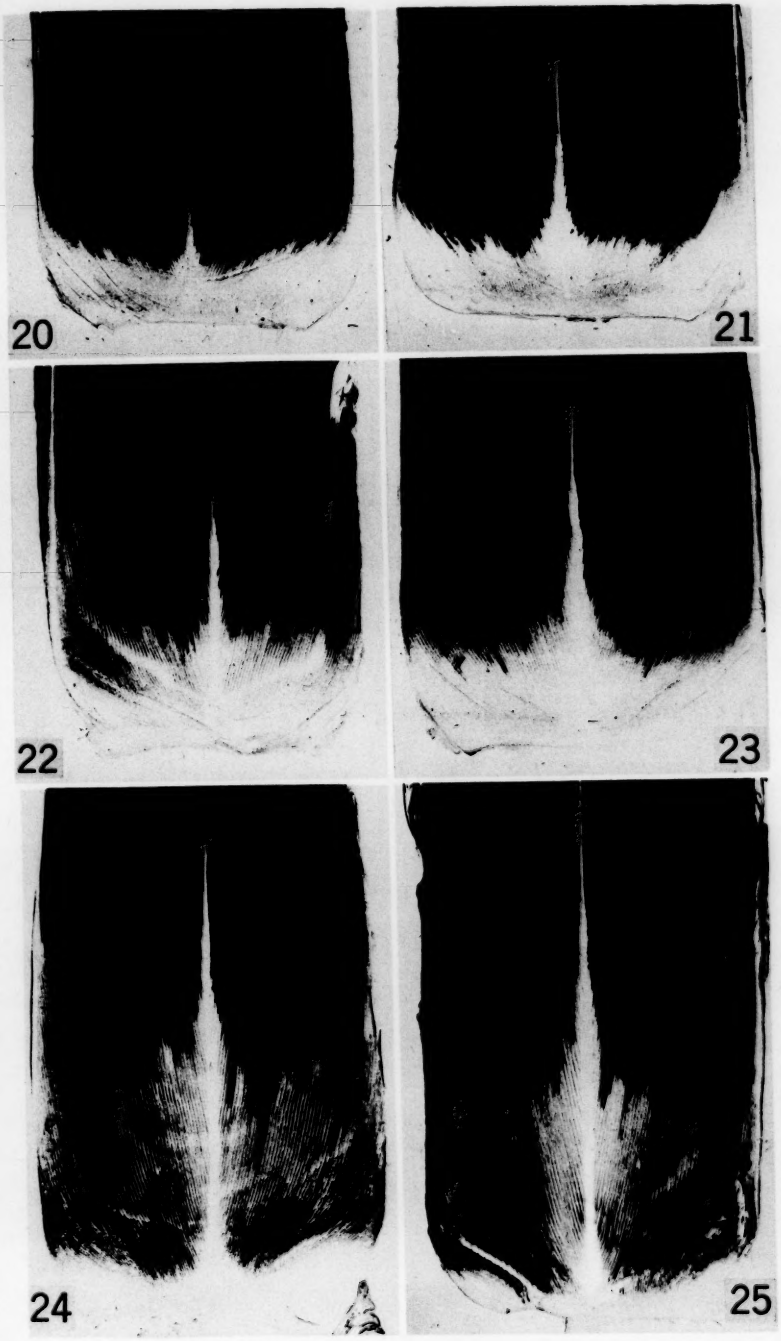

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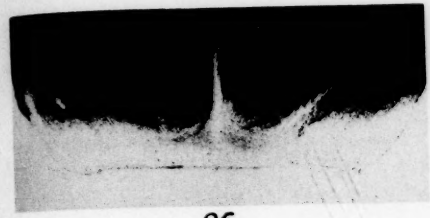
PLATE V



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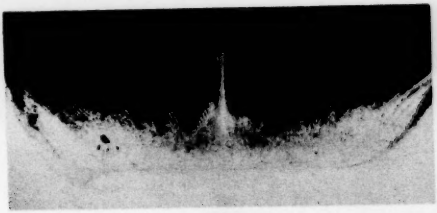

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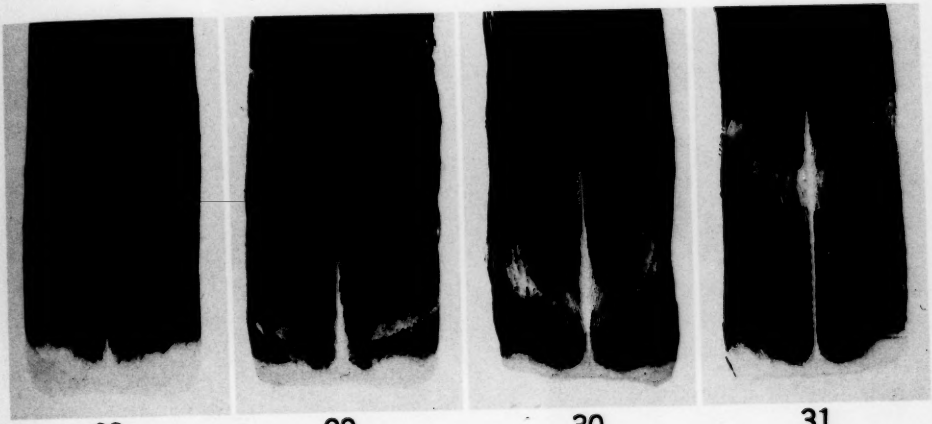

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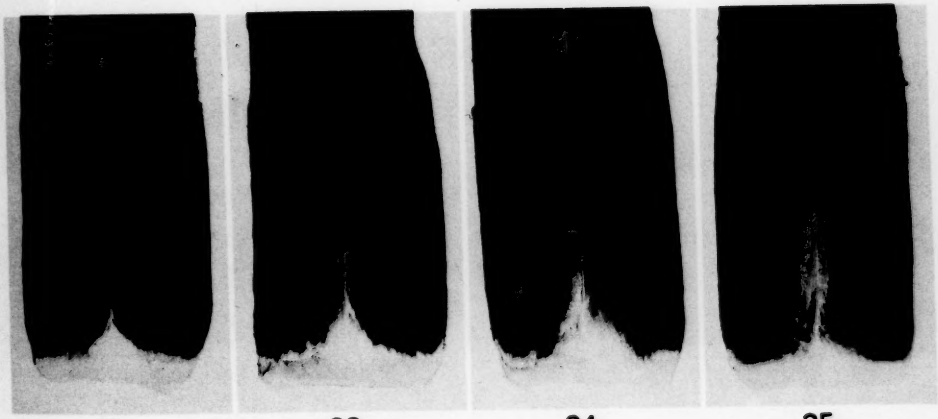

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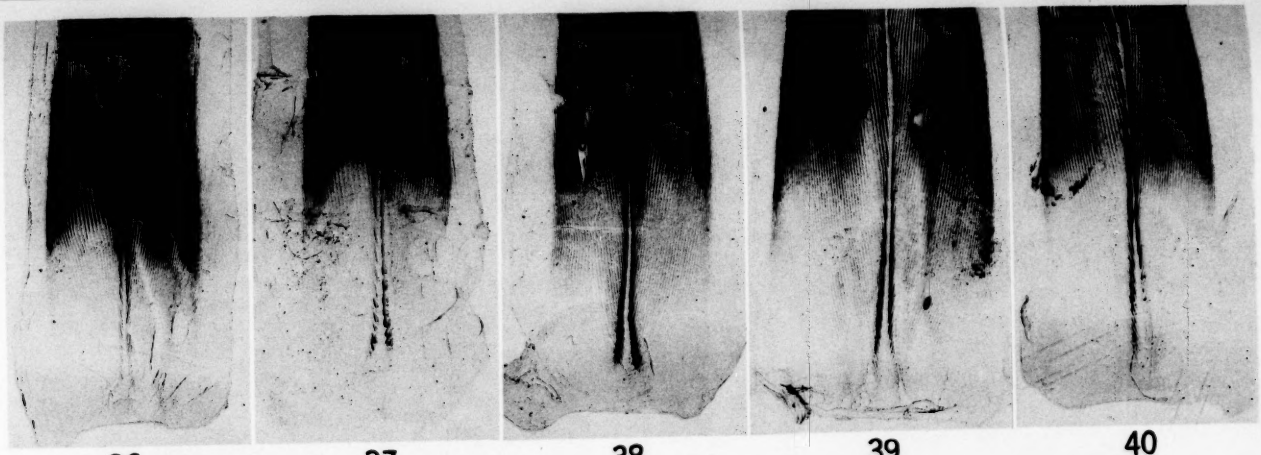

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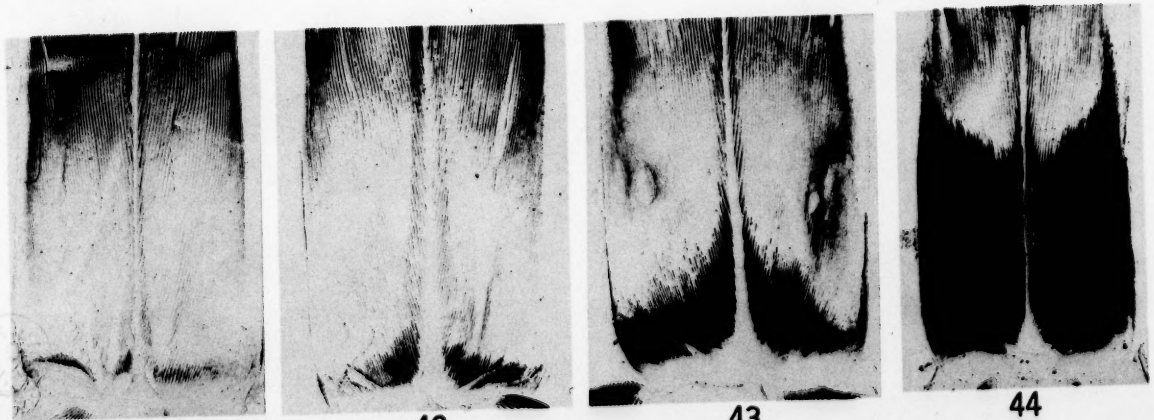

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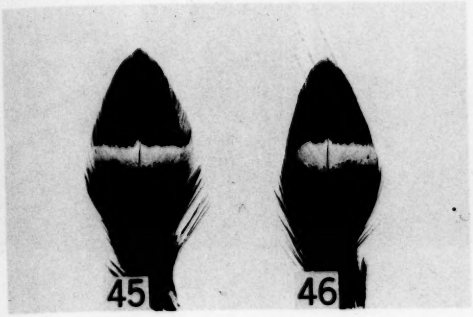
PLATE VI

PLATE VII



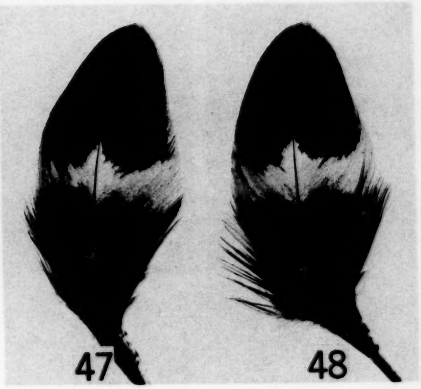
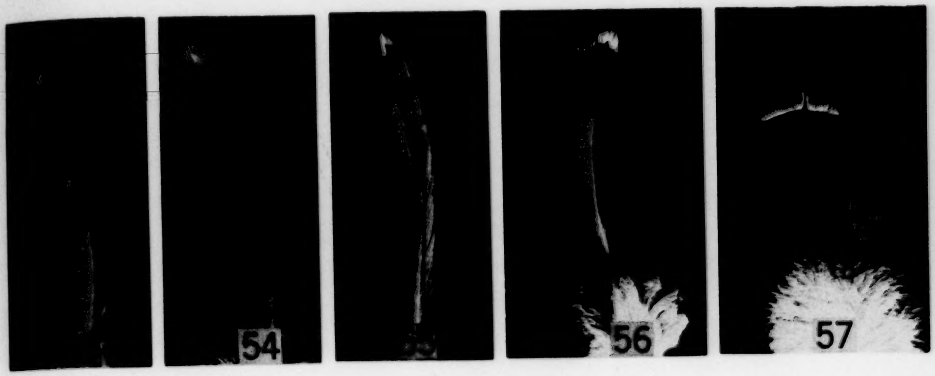
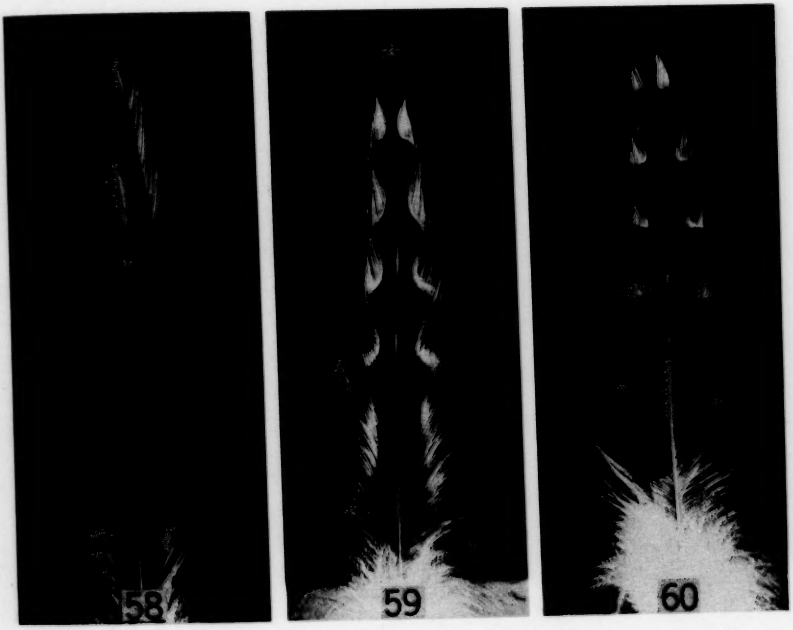



PLATE VIII